



An Integrative Taxonomy of Amphibians of Nepal: An Updated Status and Distribution

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Abstract Amphibians are declining worldwide. At the same time, each year many species of amphibians are described, indicating that biological diversity on Earth remains poorly understood. This is especially true for mountainous regions, such as the Himalayas, which are also regarded as a biodiversity hotspot. However, it is most likely that the current diversity of amphibians in Nepal is highly underestimated. Amphibian inventories by the past researchers primarily used phenotypic characters as the basis for species identification. However, the application of molecular systematics methods to taxonomic studies has successfully uncovered the taxonomic status of several cryptic taxa. This study used both molecular (16S rDNA sequences) and morphological comparisons and estimated the diversity of amphibians from Nepal. This study reports the occurrence of 38 species of amphibians based on both molecular and morphological comparisons. Among them, three species were recorded for the first time in Nepal. This study also invalids the taxonomic status of *Sphaerotheca paschima*. This study highlights the importance of integrative taxonomic approaches that help to resolve the current taxonomic ambiguities of Nepalese amphibians.

1. Introduction

Amphibians are considered the most threatened group of vertebrates on the planet (Hoffmann *et al.*, 2010). The global amphibian population is declining at an alarming rate and habitat loss, for instance, is considered to be one of the main factors that can lead to the biodiversity crisis of amphibian communities (Houlahan and Findlay, 2003; Gallant *et al.*, 2007; Hillers *et al.*, 2008). Moreover, recent climate change (Araújo *et al.*, 2006) and environmental pollution (Davidson, 2004) have further threatened the distribution and community composition, and even local extinction of amphibian species. Many amphibians are still undescribed from different geographical locations. As a result, amphibians have the highest rate of new species discoveries than other vertebrates (Köhler *et al.*, 2005) and more than 150 new species been described alone in 2016 (Amphibiaweb, 2020). Although, species descriptions of amphibians have been increasing in recent years, however, they suffer the highest extinction rate among all vertebrates (Stuart *et al.*, 2004). This is a fact that many amphibian species are possibly extinct before they described (Costello *et al.*, 2013). Therefore, a basic understanding of biological diversity is urgently needed. As taxonomy is the basic biological discipline refers to “the theory and practice of classifying organisms” (Mayr, 1969) and is a systematic reconstruction and examination of the past biological activities such as biogeographic linkage and adaptive radiation. It is also regarded as a fundamental aspect of species conservation and management (Mace, 2004). Therefore, taxonomic research should receive priority in regions where the identity of species is poorly known. Several efforts have been made by earlier biologists and explorers to describe the species and reported that only 14.2% of species have been described from the earth and 11.3% from the ocean (Camilo *et al.*, 2011). However, it is an

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extremely notorious difficult task to ascertain and explore the number of species in the remote terrain such as the Himalayas. Nepal possesses high biodiversity but is perhaps the most poorly studied country in the region, especially in terms of amphibian diversity.

Nepal supports a high unrecognized anuran diversity (Schleich and Kästle, 2002). Currently, Nepal harbours more than 52 species of amphibians (Shah and Tiwari, 2004), but information about them is generally derived from relatively old studies (Smith and Battersby, 1953; Nanhoe and Ouboter, 1987; Mitchell and Zug, 1995; Zug and Mitchell, 1995). These records are primarily based on the species already discovered earlier from neighbouring countries like India and Tibet in China. Available data on Nepalese amphibians suggested that more than 75% of amphibians are categorized as “Least Concern” in the IUCN Red Data List (IUCN, 2001). However, these numbers are not based on detailed field studies of population densities within Nepal but are either from presence-only surveys or from anecdotal information. This further confirmed that Nepalese amphibians are poorly understood and many of them are incorrectly classified (Khatiwada *et al.*, 2017).

Previous studies on Nepalese amphibians were mainly focused on their taxonomy, geographic distribution, behaviour, developmental biology, natural history, and conservation (Dubois, 1974b; Nanhoe and Ouboter 1987; Shrestha, 1989). Species identifications were mainly based on traditional morphological comparisons (Dubois 1974a, 1974b; Dubois and Matsui, 1983). However, small-bodied and highly cryptic frogs are extremely difficult to diagnose using only morphological

information (Matsui *et al.*, 2005). Cryptic frogs with high morphological similarity further create uncertainty in species identification, especially in the field (Khatiwada *et al.*, 2017). With the advancement of molecular technology in taxonomic research, diagnosing cryptic taxa has become easier (Taylor and Harris, 2012). Recently, molecular phylogenetics has become an indispensable tool for the identification of species origins, demographic changes, species migration patterns and conservation (Yang and Rannala, 2012). The continuing description of new amphibians is the result of the introduction of molecular methods in the taxonomic study in south Asia (Khatiwada *et al.*, 2015; Khatiwada *et al.*, 2017; Khatiwada *et al.*, 2019a).

However, species taxonomy using molecular methods in Nepal is greatly lacking. Although, historical compilations of species by different researchers described above and recent field studies greatly improved the knowledge on Nepalese amphibians. For the very first time, this study used molecular and morphological information of amphibians to ascertain their taxonomic position from Nepal.

2. Materials and Methods

2.1. Sampling and data collection This study was carried out between May to September from 2014 to 2018 at several sites in the eastern, central, western and far-western part of Nepal Himalaya extends between 80°04'–88°12' E and 26°22'–30°27' N (Figure 1). Amphibians were surveyed using visual encounter surveys, acoustic surveys, and leaf litter searches during at

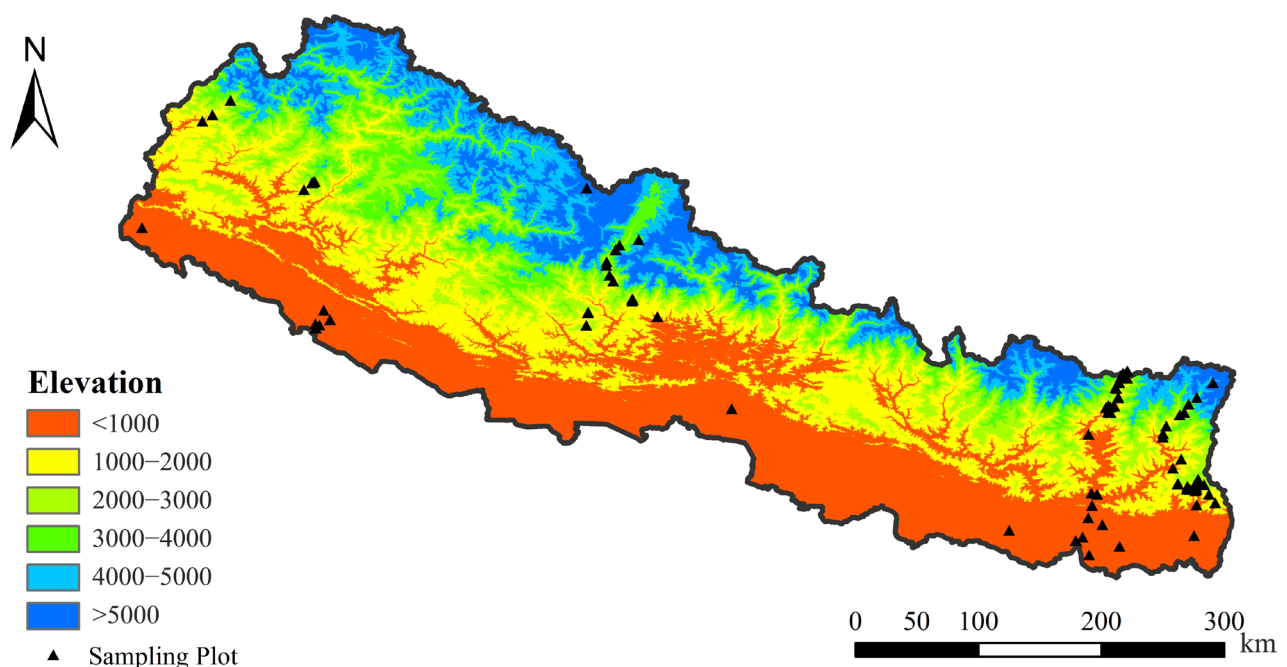


Figure 1 Map of Nepal showing sampling locations.

night (19 : 00–00 : 00) (detailed survey methods can be found in: Khatiwada *et al.*, 2016, 2019b). Specimens were collected by hands, euthanized, tissue samples collected and fixed in 4% formalin for 24 hrs, then preserved in 70% ethanol. Tissue samples were taken from thigh muscle then preserved to 95% ethanol for further molecular analysis. Morphological

measurements were carried out using digital calliper (to nearest 0.01 mm). Measurement descriptions are provided in Table 1. Preserved specimens were also measured in the herpetological museum of Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China *Collection des Reptiles et Amphibiens* of Muséum national d'Histoire naturelle (MNHN), Paris, France,

Table 1 Morphological characters used and their measurement descriptions for adults and tadpoles.

Abbreviation	Morphology	Measurement description
Adult morphology		
SVL	Snout vent length	Distance from tip of snout to posterior edge of vent
HL	Head length	Distance from angle of jaws and snout-tip
HW	Head width	Measured at posterior angle of jaws
SL	Snout length	From tip of snout to anterior corner of eye
SN	Nostril to the tip of the snout	Distance from nostril to tip of snout
ED	Eye diameter	Horizontal diameter of eye
IND	Internarial distance	Minimum distance between external nares
ENL	Eyelid-naris distance	Minimum distance between eyelid and rim of naris
UEW	Upper eyelid width	Greatest width of upper eyelid
IOW	Inter orbital width	Minimum distance between upper eyelids
TYD	Tympanum diameter	Largest tympanum diameter
TYE	Tympanum orbit distance	Distance from anterior border of tympanum to posterior orbital border
FAL	Forearm length	Distance from elbow to base of outer metacarpal tubercle
HAL	Length of hand	Measured from base of outer metacarpal tubercle to tip of third finger
F1	First finger length	Distance from base of second finger to tip of first finger
F2	Second finger length	Distance from base of first finger to tip of second finger
F3	Third finger length	Distance from base of second finger to tip of third finger
F4	Fourth finger length	Distance from base of third finger to tip of fourth finger
THL	Thigh length	Distance from groin to knee
SHL	Shank length	Distance from knee to heel
TL	Tarsus length	Distance from heel to inner metatarsal tubercle
FOL	Foot length	Distance from inner metatarsal tubercle to tip of fourth toe
T1	First toe length	Maximum length from base of first subarticular tubercle first toe tip
T2	Second toe length	Maximum length from base of first subarticular tubercle second toe tip
T3	Third toe length	Maximum length from base of first subarticular tubercle third toe tip
T4	Fourth toe length	Maximum length from base of first subarticular tubercle fourth toe tip
T5	Fifth toe length	Maximum length from base of first subarticular tubercle fifth toe tip
Tadpole morphology		
TL	Total length	Distance from tip of snout to tip of tail
BL	Body length	Distance from tip of snout to junction of posterior body and tail musculature
BW	Body width	Highest body width
BH	Body height	Highest body height
HW	Head width	Measured at posterior angle of the jaws
TAL	Tail length	Measured from junction of posterior body the tail musculature to tail tip
TMH	Tail muscle height at base of tail	Maximum tail muscle height
UTF	Upper tail fin height	Maximum height of upper fin, from upper margin of the tail musculature to upper margin of upper fin
LTF	Lower tail fin height	Maximum height of the lower fin, from lower margin of lower fin to lower margin of tail musculature
TMW	Tail muscle width at base of tail	Maximum tail muscle width
MTH	Maximum height of tail	Maximum height of the tail
ENL	Eyelid-naris distance	Minimum distance between eyelid and rim of naris
SN	Nostril to the tip of the snout	Distance from the nostril to the tip of the snout
SSD	Snout-spiracle distance	Distance from tip of snout to posterior margin of spiracle
IND	Internarial distance	Minimum distance between external nares
IOW	Inter orbital width	Minimum distance between upper eyelids
ED	Eye diameter	Horizontal diameter of eye

the British Museum of Natural History (BMNH), UK and Central Department of Zoology, Tribuvan University (CDZ-TU) (for details see: Khatiwada *et al.*, 2015; Khatiwada *et al.*, 2017; Khatiwada *et al.*, 2019a; Khatiwada *et al.*, 2020).

2.2. Molecular methods Total genomic extraction was carried out using the DNeasy Tissue Kit (QIAGEN). A DNA fragment of the mitochondrial 16S rRNA gene was amplified with the primers and polymerase chain reaction (PCR) conditions provided in (Khatiwada *et al.*, 2017). The amplified PCR products were purified using a Qiagen PCR purification kit and sequenced were obtained from an ABI 3100 automated sequencer. All sequences were deposited in GenBank under accession numbers (MT982964–MT983198). Available nucleotide sequences of 16S gene of amphibian species were downloaded from the NCBI GenBank database and aligned with ClustalW built into BIOEDIT Version 7.1.9 (Thompson *et al.*, 1997) using the default parameters. Alignments were also checked and manually edited, if necessary. Maximum Likelihood (ML) analysis was conducted with the rapid bootstrapping algorithm using the program RAxML v8.00 (Stamatakis, 2014) on the CIPRES Science Gateway server v3.2 (Miller *et al.*, 2010). Uncorrected k2p pair-wise distances within and across genus for 16S gene sequences were calculated with MEGA7 (Kumar *et al.*, 2016).

3. Result

This study produced 16S DNA barcode data for 235 specimens, representing 38 species of amphibians belonging to two orders and seven families (Table 2). Among them, the highest number of species were recorded from the family Dicroglossidae ($n =$

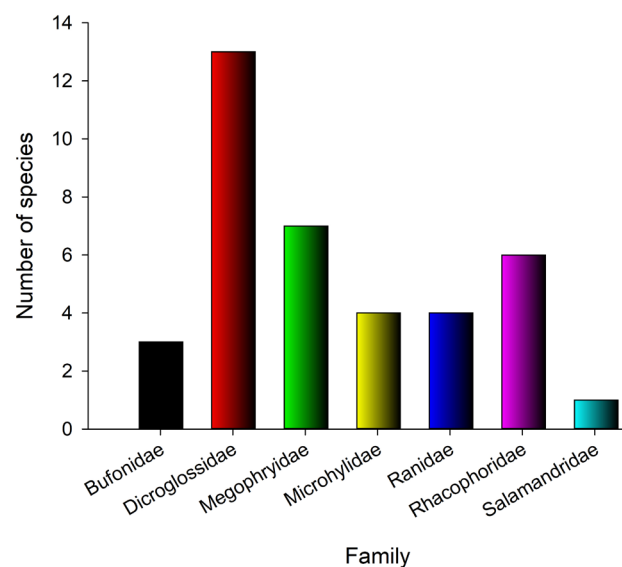


Figure 2 The number of amphibian species recorded in the study area.

13) followed by Megophryidae ($n = 7$) and Rhacophoridae ($n = 7$) (Figure 2). We also provided brief accounts for each species identified with morphological features.

Family: Bufonidae (Gray, 1825)

Molecular data. Based on the molecular data, three lineages of *Duttaphrynus* frogs have been identified from Nepal. *Duttaphrynus* species were suggested to form a monophyletic group based on both ML analysis (Figure 3). The phylogenetic analysis showed that *Duttaphrynus* samples from Nepal were 99%–100% similar based on 16S data with the type series of *Duttaphrynus melanostictus* (KJ697684) from India orientali (Dutta and Das, 2013). *D. melanostictus* was the sister taxon to *D. brevivirostris* and *D. parietalis*. The uncorrelated genetic divergence

Table 2 The list of the amphibians recorded from 2014 to 2018.

Order	Family	Species	Common name
Anura	Bufonidae	<i>Duttaphrynus himalayanus</i>	Himalayan toad
		<i>Duttaphrynus melanostictus</i>	Southeast Asian toad
		<i>Duttaphrynus stomaticus</i>	Indus valley toad
	Dicroglossidae	<i>Euphlyctis kalasgramensis</i>	Kalasgram skittering frog
		<i>Hoplobatrachus crassus</i>	Jerdon's bullfrog
		<i>Hoplobatrachus tigerinus</i>	Indian bullfrog
		<i>Minervarya nepalensis</i>	Nepal cricket frog
		<i>Minervarya pierrei</i>	Pierrei cricket frog
		<i>Minervarya syhadrensis</i>	Syhadra frog
		<i>Minervarya teraiensis</i>	Madhese kithre bhyaguto
		<i>Minervarya orissaensis</i>	Orissa cricket frog
		<i>Nanorana blanfordii</i>	Blanford's paa frog
		<i>Nanorana liebigii</i>	Liebig's paa frog
		<i>Nanorana rostandi</i>	Rostand's paa frog
		<i>Sphaerotheca maskei</i>	Maske's frog
		<i>Sphaerotheca swani</i>	Swani's frog
	Megophryidae	<i>Megophrys monticola</i>	Mountain horned frog
		<i>Megophrys robusta</i>	Robust spadefoot toad
		<i>Megophrys zhangii</i>	Zhang's horned toad
		<i>Scutiger boulengeri</i>	Xizang alpine toad
		<i>Scutiger nepalensis</i>	Nepal lazy toad
		<i>Scutiger sikkimensis</i>	Sikkim high altitude toad
		<i>Scutiger ghunsa</i>	Ghunsa high altitude toad
	Microhylidae	<i>Microhyla taraiensis</i>	Tarai narrow-mouthed Frog
		<i>Microhyla nilphamariensis</i>	Narrow-mouthed Frog
		<i>Uperodon taprobanicus</i>	Sri Lankan bullfrog
		<i>Uperodon systoma</i>	Marbled balloon frog
	Ranidae	<i>Hydrophylax leptoglossa</i>	Cope's Assam frog
		<i>Amolops formosus</i>	Assam torrent frog
		<i>Amolops nepalicus</i>	Nepal torrent frog
		<i>Amolops mahabharatensis</i>	Mahabharat torrent frog
Rhacophoridae	Polypedates	<i>Polypedates maculatus</i>	Common tree frog
		<i>Polypedates taeniatus</i>	Six-lined tree frog
		<i>Polypedates teraiensis</i>	Tarai tree frog
		<i>Polypedates himalayensis</i>	Himalayan tree frog
	Raorchestes	<i>Raorchestes amandalii</i>	Himalayan foam nesting frog
		<i>Zhangixalus smaragdinus</i>	Giant tree frog
Caudata	Salamandridae	<i>Tylototriton himalayanus</i>	Himalayan salamander

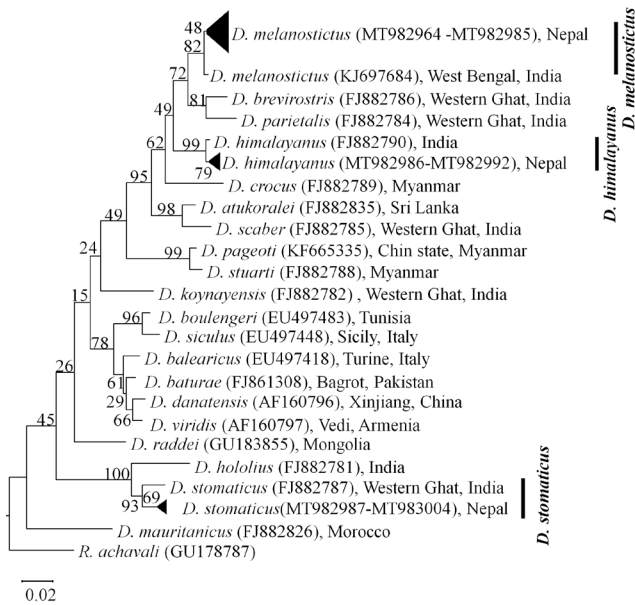


Figure 3 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships among 21 species of the genus *Duttaphrynus*. Numbers present on branches of ML tree are bootstrap support values for Maximum likelihood. Genbank accession number are presented in parenthesis.

between *D. melanostictus* with *D. brevivirostris* and *D. parietalis* were 3.7% and 4.2%.

Duttaphrynus species from above 1800 m elevation were 96%–99% similar based on 16S data with the type series of *D. himalayanus* (FJ882790) from India (Van Bocxlaer *et al.*, 2009).

D. himalayanus is the sister taxon of *D. crocus* (FJ882789) from Myanmar and the uncorrelated genetic divergence between them was 7.7%–6.7%.

Similarly, species from Jabdi, Sunsari district eastern Nepal were 91%–86% similar based on 16S data with the type series of *D. stomaticus* (FJ882787) from Western Ghats, India (Van Bocxlaer *et al.*, 2009). *D. stomaticus* was sister taxon of *D. hololius* (FJ882781) and the uncorrelated genetic divergence between them was 5.6%–5.5%.

***Duttaphrynus melanostictus* (Schneider, 1799) (Figure 4A)**

Common name: Asian Common Toad

Nepali name: Khasre bhyaguto

Description: Body size (SVL) in males 41–71 mm ($n = 33$) and 60–144 mm ($n = 14$) in females

Head wider than long with distinct rostral, preorbital, supraorbital, postorbital and a short orbito-tympanic and cranial crest. The inter-orbital distance broader than upper eyelid, tympanum very distinct and round, contacting to the posterior end of the postorbital ridge. Eyes round, pupil horizontal. Parotoid glands are kidney-shaped or elliptical and covered with warts having black tips. The first and second fingers subequal; the relative length of fingers $2 < 1 < 4 < 3$. Fingers with double subarticular tubercles. Finger and toe tips round. The relative toes length $1 < 2 < 5 < 3 < 4$, partially webbed. Toes with single subarticular tubercle. Tibiotarsal articulation reached the tympanum when the hindlimb is kept parallel to the body. Breeding males with sub-gular vocal sacs and nuptial pads



Figure 4 A: *Duttaphrynus melanostictus* B: *D. stomaticus*; C: *D. himalayanus* collected from Kimathanka, Sankhuwasava, Nepal; D: *D. himalayanus*. Photo by Janak Raj KHATIWADA.

on the inner side of first and second fingers. Skin rough and heavily tuberculated on flanks, tubercles usually tipped with dark brown spines. Ventral dirty white and granular. The throat of the breeding male light orange or yellow.

Natural history notes: All individuals were found in the ground, holes, and crevices, under the rock and leaf litter. This toad is lethargic and often nocturnal. In Nepal, it is the most common amphibian, found in and around the human settlement. They are active soon after sunset. They primarily feed on ants and other insects (Khatiwada *et al.*, 2016). In hot subtropical environments of lower elevational zones of Tarai, Nepal, the breeding is from late February to August.

Distribution: Widely distributed in the mid-hill and lower Tarai regions (below 1800 m) of Nepal.

***Duttaphrynus stomaticus* (Lütken, 1864)** (Figure 4B)

Common name: Marble toad, Assam toad

Nepali name: Khasre bhyaguto

Description: Body size (SVL) in males 70–80 mm ($n = 4$) and 49–58 mm ($n = 6$) in females

The body is an oblong oval. The head is broader than long, snout is pointed. Head flat and slightly concave. Canthus rostralis rounded to angular, lacking cranial crests; interorbital space little broader than the upper eyelid. Tympanum distinct and oval located at the posterior corner of the eye, the size (diameter of tympanums) about the two third of eye. Parotid glands elliptical and flat, longer than broad. The first and second fingers subequal; the relative length of fingers $2=4<1<3$. Toes and fingertips round. The relative toes length $1<2<5<3<4$, partially webbed, tibiotarsal articulation reached the between shoulder and eye when the hindlimb is kept parallel to the body. Dorsum with small, rounded and flat warts with black tips. The ventral of hands, palm and legs with numerous creamy white warts with black tips. Dorsum light gray to olive or yellowish green to almost black. Ventrums a uniform dirty whitish to yellowish colour. Upper lip creamy. Tips of digits dark brown. Males with subgular vocal sac.

Natural history notes: All individuals were found in the ground, holes, and crevices, under the rock and leaf litter. This toad is mostly nocturnal. They primarily feed on ants and other insects (Khatiwada *et al.*, 2016). In hot subtropical environments of lower elevational zones of tarai, Nepal, the breeding is initiated from late February to August. It breeds into shallow water either standing or running, rice fields and ditches. The breeding occurs after the first showers of monsoonal rains in Nepal.

Distribution: Widely distributed in the lower tarai regions (below 400 m) of Nepal.

***Duttaphrynus himalayanus* (Günther, 1864)** (Figures 4 C, D)

Common name: Himalayan Toad

Nepali name: Himali khasre bhyaguto

Description: Body size (SVL) in females 74–105 mm ($n = 10$) and 50–71 mm ($n = 10$) in males.

Head wider than long, deeply concave. Snout short and blunt. Nares nearer to the tip of snout than to eye. Interorbital distance broader than the diameter of upper eyelid. Tympanum very small or indistinct; round, two third of the size of the eye. The parotid glands elliptical and flat, almost the size of head length. The relative length of fingers $2<1<4<3$. Fingers are free of webbing. Two large plumber tubercles, outer tubercle round and about two to two and a half size of ovoid inner tubercle. Toes and fingertips rounds. The relative toes length $1<2<5<3<4$, fully webbed, two oval meta-tarsal tubercles, tibiotarsal articulation reached the between eyes and tip of snout when the hindlimb is kept parallel to the body. Dorsum with irregular small, rounded and flat warts with black tips. The ventral of hands, palm and legs with numerous creamy white warts with black tips. Dorsum greyish brown to brown or yellowish or almost black. Ventrums a uniform dirty whitish to yellowish colour. Specimens from Kimathanka and Hatiya (Makalu Barun National Park) had black and creamy white blotches on their ventrum.

Natural history notes: All individuals were observed in the cleared forest, walking trails, man-made and natural lakes and under leaf litter. This toad is mostly nocturnal. In Nepal, the breeding is initiated from late April to August. It breeds into shallow water either standing or running, rice fields and ditches.

Distribution: Widely distributed in the temperate region (below 1700 to 3000 m) of central and eastern Himalayas.

Family: Dicroglossidae (Anderson, 1871)

Genus: *Euphlyctis*

Molecular data. The phylogenetic analysis showed that *Euphlyctis* samples from Nepal were 94%–96% similar based on 16S data with the type series of *E. kalasgramensis* (KP091866) from Kalasgram, Barisal, Bangladesh (Howlader *et al.*, 2015). *E. kalasgramensis* was the sister taxon to *E. cyanophlyctis* (AB167938) from Madikeri, Western Ghats, India, and the uncorrelated genetic divergence between them was 4.5%–5.1% (Figure 5).

Euphlyctis kalasgramensis (Howlader, Nair, Gopalan, and Merilä, 2015) (Figure 6).

Common name: Skittering frog

Nepali name: Bhiase bhyaguto

Description: Body size (SVL) in females 41–62 mm ($n = 8$) and 30–45 mm ($n = 20$) in males

Head broader than wide, snout pointed, canthus rostralis indistinct, nostrils much closer to tip of snout than to eyes. Interorbital distance broader than the diameter of upper eyelid. Tympanum round and size almost half of eye. The relative length of fingers $1=2<4<3$. Fingers are free of webbing. Single

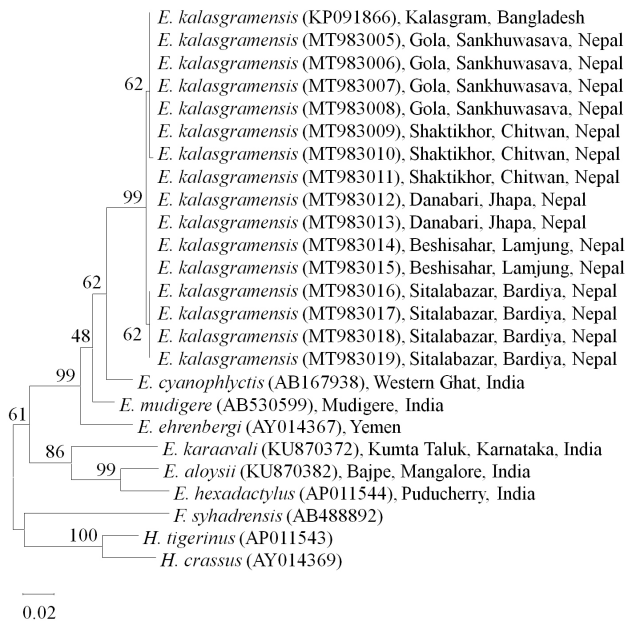


Figure 5 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the genus *Euphlyctis*. Numbers present on branches are bootstrap support values for Maximum likelihood. Genbank accession numbers are presented in parenthesis.

and rounded subarticular. Fingers devoid of dermal ridge. Toes and fingertips rounds. The relative toes length $1 < 2 < 3 < 4$, fully webbed, two oval meta-tarsal tubercles, elongated and compressed inner metatarsal tubercle. Oval and small outer metatarsal tubercle. Dorsal surface of the body grayish-brown with darker rounded spots. Limbs with incomplete dark bands. Ventral surface white. For more details refer to Howlader *et al.* (2015).

Natural history notes: All individuals were observed in temporary or permanent pools, rice, and crop fields. This species is mostly nocturnal. In Nepal, the breeding is from March to August. It breeds into shallow water either standing or running, rice fields and ditches.

Distribution: Widely distributed in Nepal (below 2500 m).

Genus: *Hoplobatrachus*

Molecular data Based on the molecular data, two lineages of *Hoplobatrachus* frogs have been identified from the Nepal. *Hoplobatrachus* species were suggested to form a monophyletic group based on both ML analysis (Figure 7). The phylogenetic analysis showed that *Hoplobatrachus* samples from first lineage were 98%–100% similar based on 16S data with the type series of *H. tigerinus* (KP091866–Mymensingh, Bangladesh and AB272594–Padil, India) (Alam *et al.*, 2008). *H. tigerinus* was the sister taxon to *H. rugulosus* (AB272596) from Nong Khai, Thailand and *H. litoralis* (AB671173) from Cox's Bazar, Bangladesh and the uncorrelated genetic divergence between them were 3.7%–4.1% and 4.1%–6.2%, respectively.

Hoplobatrachus species from second lineage were 96%–99% similar based on 16S data with the type series of *Hoplobatrachus crassus* (AB290413) from Assam, India and *Hoplobatrachus crassus* (AB272595) Khulna, Bangladesh (Alam *et al.*, 2008). *H. crassus* was the sister taxon of *H. occipitalis* (AY014373) from Myanmar and the uncorrelated genetic divergence between them was 7.7%–6.7%.

Hoplobatrachus crassus (Jerdon, 1853) (Figures 8 A and B)

Common name: Jerdon's bullfrog

Nepali name: Sigare paha

Description: Body size (SVL) in females 55–84 mm ($n = 21$) and

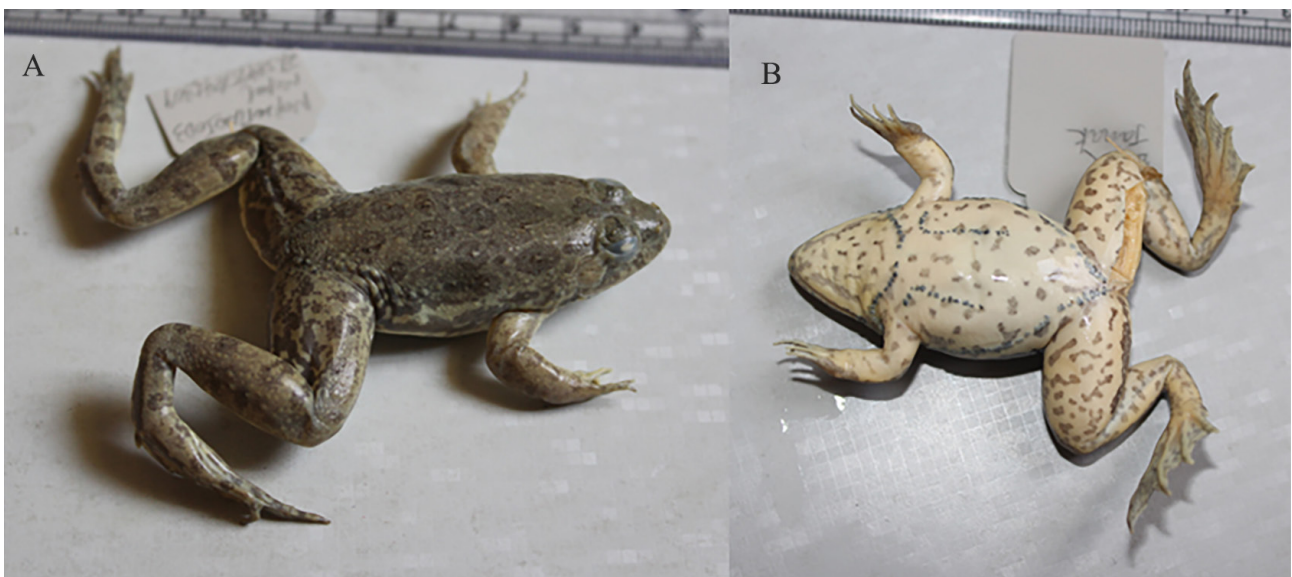


Figure 6 A: dorsal view of *Euphlyctis kalasgramensis* (in preservative). B: ventral view. Photo by Janak Raj KHATIWADA.

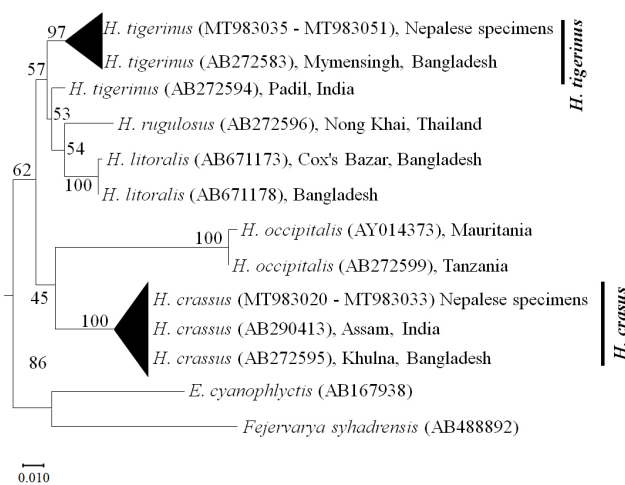


Figure 7 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships among the genus *Hoplobatrachus*. Numbers present on branches of ML tree are bootstrap support values for Maximum likelihood. Genbank accession numbers are presented in parenthesis.

50–75 mm ($n = 11$) in males

Head pointed, longer than wide, snout pointed and slightly extended over the lower jaw. The canthus rounded and loreal region concave. The nostrils much closer to tip of snout than to the anterior corner of eyes. Interorbital distance is almost equal to the diameter of upper eyelid (female: IOD = 3.5 and WUE = 3.3; male: IOD = 3.4 and WUE = 2.8). Tympanum round and

size almost half of eye. The relative length of fingers $1=2<4<3$. Fingers with dermal fringe and free of webbing. Single, ovoid metacarpal tubercle in first finger and rounded palmar tubercle. The relative toes length $1<2<5<3<4$, fully webbed. Lateral margins of 1st and 5th toe possessed dermal fringes. Inner metatarsal tubercle is shovel shaped and almost 60% of the length of 1st toe. Tibiotarsal articulation reached only to the tympanum when the hindlimb is kept parallel to the body.

Dorsal surface yellowish green to olive or greyish brown with irregular dark spots. Dorsum smooth to slightly granular with discontinuous longitudinal folds. Flank of the body grayish-brown with darker rounded spots. Limbs with incomplete dark bands with minute white warts. Ventral surface of the limbs usually dirty white in colour, throat molted with black and belly dirty white. Females are distinctly larger than males. Breeding males with sub-gular vocal sacs and nuptial pads on inner side of first finger and metacarpal tubercle.

Natural history notes: It is a semi-aquatic lowland species and most found in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the breeding is after the first monsoonal rainfall at the end of May or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Widely distributed in Nepal (below 400 m).

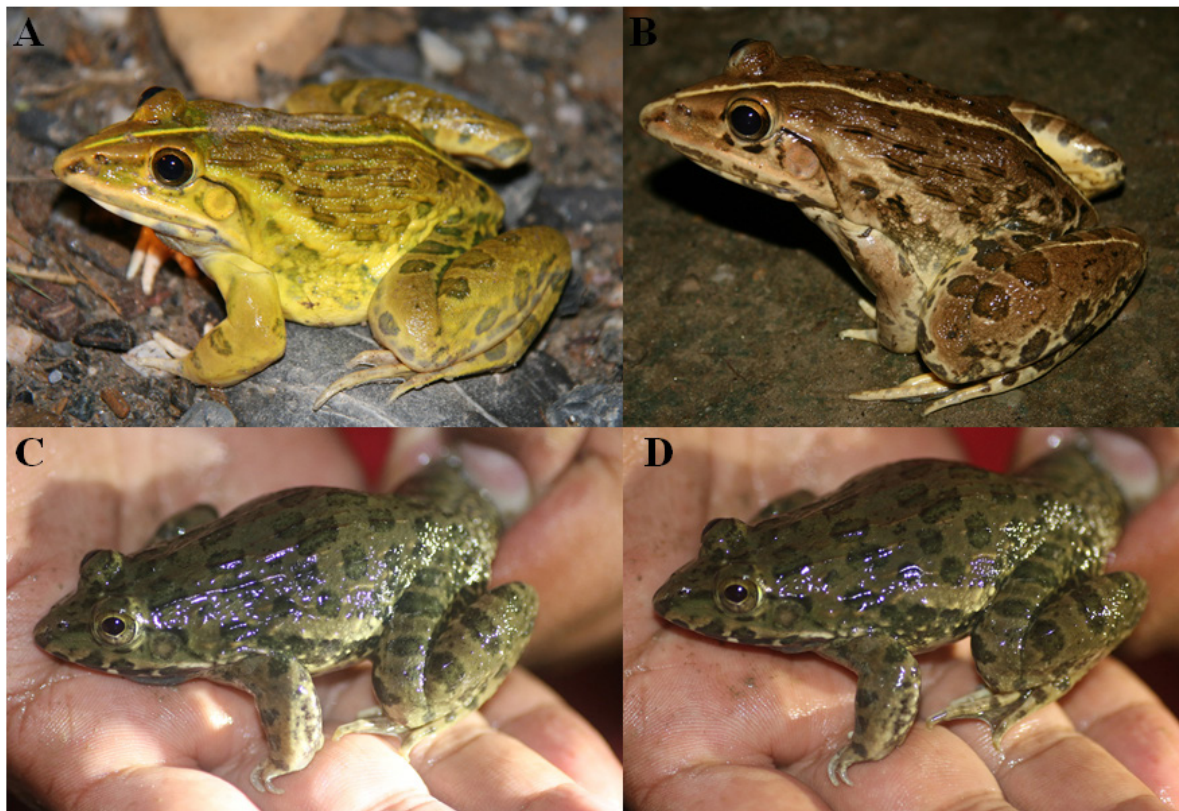


Figure 8 A and B: dorsal view of *Hoplobatrachus crassus*. C and D: dorsolateral view of *H. tigerinus*. Photo by Janak Raj KHATIWADA.

***Hoplobatrachus tigerinus* (Daudin, 1802)** (Figures 8 C and D)

Common name: Tiger frog

Nepali name: Sigare paha

Description: Body size (SVL) in females 55–113 mm ($n = 27$) and 49–135 mm ($n = 19$) in males

Head pointed, longer than wide, snout pointed, canthus obtuse; loreal oblique, slightly concave. Nares oval and dorsally located, much closer to tip of snout than to the anterior corner of eyes. Interorbital distance almost equal to the diameter of upper eyelid (female: IOD = 4.1 and WUE = 3.9; male: IOD = 5.1 and WUE = 4.9). Tympanum round and almost 50% to 70% of the size of eye. Fingers thick and obtusely pointed. The relative length $2 \leq 4 < 3$. Fingers with dermal fringe and free of webbing. Inner and medial metacarpal tubercles flat and oval shaped. Legs long and muscular. The relative toes length $1 < 2 < 5 < 3 < 4$, fully webbed except 4th toe. The 5th toe possessed lateral dermal fringe. Inner metatarsal tubercle shovel shaped, long, flattened and blunt and found at the base of 1st toe. Outer tubercle is absent. Tibiotarsal articulation reaches the level between nares and the anterior angle of the eyes when the hindlimb is kept parallel to the body. Dorsal surface yellowish green to olive or greyish brown in colour. There is an irregular pattern of dark olive, greyish brown or irregular dark spots. The flanks yellowish green. Mid-dorsal line continuous or interrupted and passes from tip of snout to the cloaca. White or yellowish white line originated from either side of posterior corner of eyes to passes to the groin. Limbs with incomplete dark bands or stripes. Ventral surface of the limbs usually dirty or uniform white in colour, throat molted with black and belly dirty white. Females distinctly larger than males. Breeding males with blue vocal sacs that are located on the sides of throat.

Natural history notes: It is a mostly aquatic species, and most found in waterlogged paddy fields, natural or artificial ponds, wet grassland and marshes. In Nepal, the breeding season is after the first monsoonal rainfall at the end of May or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Widely distributed in Nepal (below 2200 m).

Genus: *Minervarya*

Molecular analyses: The aligned dataset of 16S contained 490 bps including 446 variable sites and 109 parsimony informative sites (excluding outgroups). Five lineages of *Minervarya* have been identified from Nepal (Figure 9). The phylogenetic analysis showed *Minervarya* samples from **Group-A** [Jabdi, Sunsari, Danabari, Jhapa (eastern Nepal), Bharatpur, Chitwan, Lete, Mustang, Damauli, Tanahu (central Nepal)] were 98%–100% similar based on 16S data with the *M. syhadrensis* (AY841747–India, and AB488892–Srilanka). *M. syhadrensis* was the sister taxon to *M. asmati* (MF322549) from Bangladesh and *M. teraiensis* from Britamod, Jhapa, Nepal and the uncorrelated

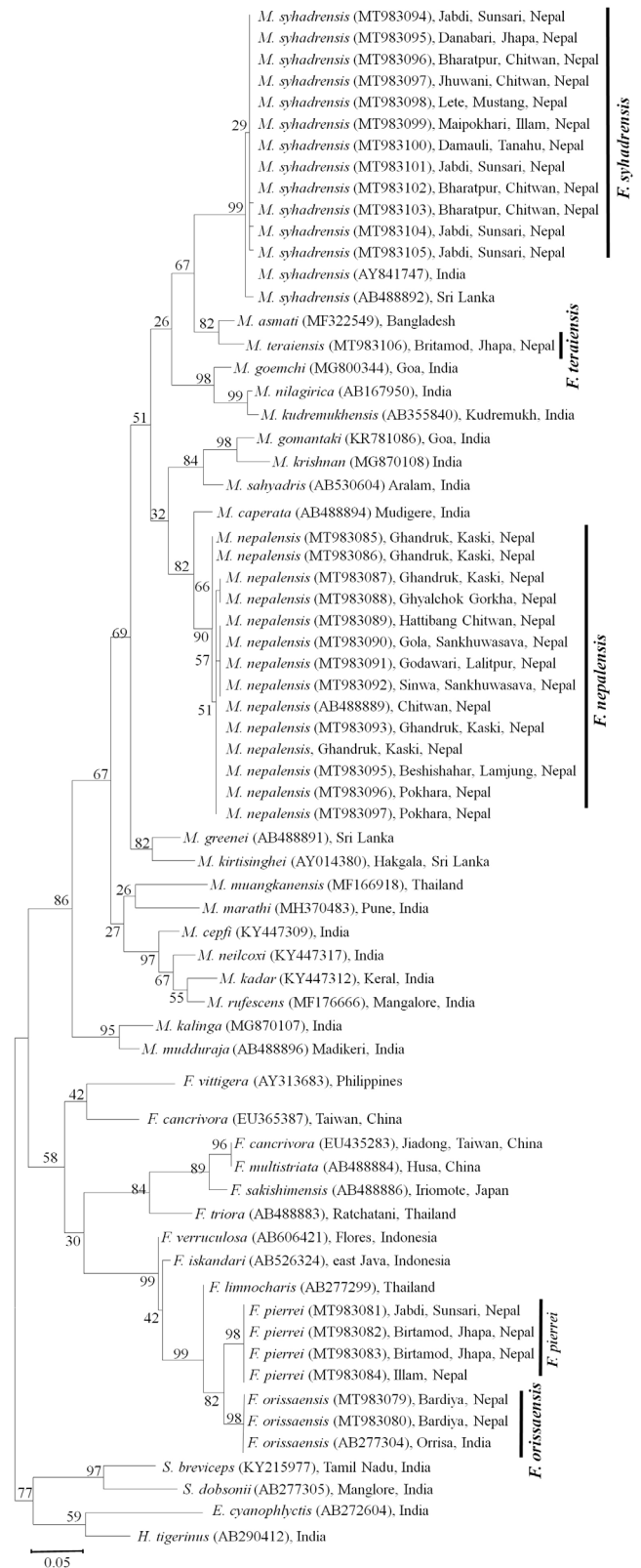


Figure 9 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships of the genus *Minervarya*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

genetic divergence between them were 5.6%–5.9% and 17.6%–17.2% respectively.

A single specimen collected from Britamod, Jhapa, Nepal (eastern Nepal–holotype locality of *M. teraiensis*) (**Group-B**) was assigned as *M. teraiensis* (for detail morphological characters: see the morphological description section for the details). *M. teraiensis* was the sister taxon to *M. asmati* (MF322549) from Bangladesh and the uncorrelated genetic divergence between them were 5.9%.

Minervarya samples from **Group-C** [Gola and Sinwa, Sankhuwasava (eastern Nepal), Ghandruk and Pokhara, Kaski, Bharatpur and Hattibang, Chitwan, and Beshishahar, Lamjung, Pokhara (central Nepal)] were 95%–100% similar based on 16S data with the *M. nepalensis* from the holotype locality–Godawari, Lalitpur, Nepal (for detail morphological characters: see the morphological description section). *M. nepalensis* was the sister taxon to *M. caperata* (AB488894) from Mudigere, India and the uncorrelated genetic divergence between them were 2.3%–2.5%.

Specimens from **Group-D** [Illam and Jabdi, Sunsari, Nepal (eastern Nepal)] were 95%–100% similar based on 16S data with the *M. pierrei* from the holotype locality–Britamod, Jhapa, Nepal, (for detail morphological characters: see the morphological description section). *M. pierrei* was the sister taxon to *F. orissaensis* (AB277304), from Orrisa, India and Bardia, Nepal and the uncorrelated genetic divergence between them were 2.0%–1.7%.

Specimens from **Group-E** [Badaiya tal, Bardiya (western Nepal)] were 100% similar based on 16S data from *F. orissaensis* (AB277304), from Orrisa, India. There was substantially low genetic divergence between *M. pierrei* vs *F. orissaensis* (AB277304) from Orrisa, India (1.5%–1.7%) and *M. pierrei* vs *F. limnocharis* (AB277299) from Thailand (1.2%–1.7%) and *F. limnocharis* (AB277299) vs *F. orissaensis* (AB277304) (1.2%).

***Minervarya syhadrensis* (Annandale, 1919) (Figure 10)**

Common name: Syhadra frog



Nepali name: Kithre bhyaguto

Description: Body size (SVL) in females 29–40 mm ($n = 3$) and 27–32 mm ($n = 5$) in males

Head pointed, longer than wide. Snout pointed and extended over the lower jaw. Canthus rostralis indistinct, loreal region is slightly concave, interorbital width almost equal to the distance between the nares, tympanum round and about half of the eye diameter. Arm shorter than length of hand, fingertips rounded, relative finger length $1=2<4<3$ and 4th finger is slightly longer than the 2nd. Subarticular tubercles globular, inner metacarpal tubercle oblong, pairs of rounded palmar tubercles. Toes webbed with rounded tips, subarticular tubercles smaller compared to those of the fingers, metatarsal tubercle very small. The tibio-tarsal articulation reaches the anterior corners of the eyes. The dorsum granulated and glandular folds are shorter. The ventrum is uniformly smooth. Dorsum colour variable from greyish to olive brownish with black irregular spots. Some of the specimens with a narrow yellowish white middorsal line. Some of the specimens with an interrupted middorsal line. The limbs with dark bands, thigh and shanks with small grey dots. The ventral sides of the limbs grey, metacarpal or metatarsal tubercles pale, toe webbing faintly marbled. Females distinctly larger than males. The males with vocal sacs.

Natural history notes: It is a mostly aquatic species and found in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the breeding is imitated from March or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Widely distributed in Nepal (170 m to 2700 m - Lete, Mustang, Nepal)

***Minervarya teraiensis* (Dubois, 1984) (Figure 11)**

Common name: Terai cricket frog

Nepali name: Madhese kithre bhyaguto

Description: Body size (SVL) in males 46.4 mm and females 53.3 mm (Dubois 1975b)



Figure 10 Dorsolateral view of *Minervarya syhadrensis* in life. Photo by Janak Raj KHATTIWADA.

Head longer than wide, snout rounded and extended over the lower jaw, cantus rostralis is rounded, loreal region concave, interorbital width equal to the distance between the nares, eye moderate, tympanum about 80% of the eye diameter. Fingers slender, free of webbing and tips rounded, relative length of fingers from shortest to longest $2 \leq 4 < 1 < 3$; oval inner metacarpal tubercle two times greater than outer median and outer metacarpal. Subarticular tubercles small, round, formula 1, 1, 2, and 1. Toes thin and small, toe tips rounded, relative length of toes $1 < 2 < 3 < 5 < 4$, webbed. Elongated and bean-shaped inner metatarsal tubercle, small rounded outer metatarsal tubercle. All five toes had small and round subarticular tubercles, formula 1, 1, 2, 3, and 2. Tibiotarsal articulation reached the posterior corner of eye when the hindlimb is kept parallel to the body. The dorsum skin is smooth with scattered tubercles arranged in rows. Some of the tubercles are orange in colour. The venter is uniformly smooth. The dorsal coloration varies from grey to greyish olive with dark irregular spots with a narrow middorsal line from the snout tip to the cloaca. The dorsum of forelimbs and hind limbs with dark bands and ventrum grey. Metacarpal and metatarsal tubercles are pale. The toe webbing is faintly marbled. The venter is creamy white with dark bars along the lower jaw. Males with a pair of gular pouches with a W-shaped dark pattern.

Natural history notes: It is a mostly aquatic species and found in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the breeding is imitated from March or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Distributed in low land eastern Nepal (180–400 m).

Minervarya nepalensis (Dubois, 1975) (Figure 12)

Common name: Nepal cricket frog

Nepali name: Neapli kithre bhyaguto

Description: SVL of males 28.0–35.5 mm and female 36.5–38.0

mm

Head wider than long, snout pointed and extended over the lower jaw, cantus rostralis is rounded, loreal region concave, interorbital width equal to the distance between the nares, eye moderate, tympanum about 75% of the eye diameter. Fingers slender, free of webbing and tips rounded, relative length of fingers from shortest to longest $2 < 1 < 4 < 3$; oval inner metacarpal tubercle and rounded outer metacarpal. Subarticular tubercles small, round, formula 1, 1, 2, and 1. Toes thin and small, toe tips rounded, relative length of toes $1 < 2 < 3 < 5 < 4$, webbed. Small elongated inner metatarsal tubercle, small rounded outer metatarsal tubercle. All five toes had small and round subarticular tubercles, formula 1, 1, 2, 3, and 2. Tibiotarsal articulation reached the anterior corner of eye when the hindlimb is kept parallel to the body. The dorsum is smooth with 4 longitudinal folds of tubercles. Several short and small rounded tubercles on the sides of the body, the dorsal surface of the tibia and the cloacal region. The venter is uniformly smooth. Dorsal surface with a white non-interrupted middorsal line passes from the tip of the snout to the cloacal region. The loreal region, the margin of lower jaw possessed dark bands. The limbs have dark stripes. The venter is near the groin is uniformly yellowish, throat and chin white in females. The margins of the lower jaw have narrow dark bars. The cloacal and ventral surface of thighs with white dots. There are pairs of lines on the lateral side of the abdomen. Males have vocal sacs and laterally dark throats.

Natural history notes: It is a mostly aquatic species and found in waterlogged paddy fields, natural or artificial ponds, wet grassland and marshes. In Nepal, the breeding is imitated from March or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Widely distributed in eastern Nepal (200–2500 m).



Figure 11 Dorsolateral view of *Minervarya teraiensis* in life. Photo by Janak Raj KHATIWADA.

***Minervarya pierrei* (Dubois, 1975) (Figure 13)**

Common name: Pierrei cricket frog

Local Name: Kithre bhyaguto

Description: Body size (SVL) in females 32–48 mm ($n = 2$) and 27–31 mm ($n = 5$) in males

Head longer than wide, snout pointed and extended over the lower jaw, cantus rostralis is rounded, loreal region concave, interorbital width equal to the distance between the nares, eye moderate, tympanum about 60% of the eye diameter. Fingers free of webbing and tips rounded, relative length of fingers

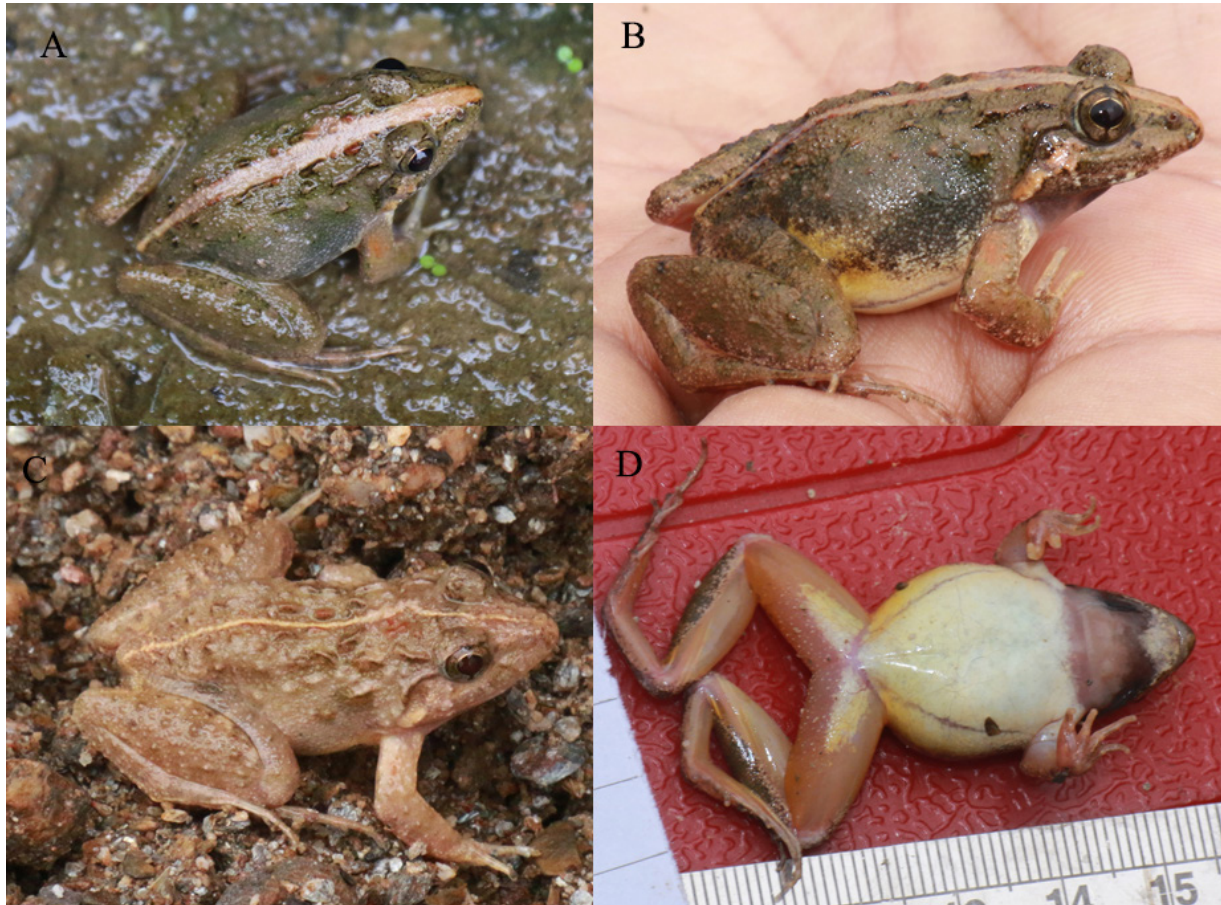


Figure 12 *Minervarya nepalensis* in life. A, B, and C: dorsolateral view; D: ventrolateral view. Photo by Janak Raj KHATIWADA.



Figure 13 Dorsolateral view of *Minervarya pierrei* in life. Photo by Janak Raj KHATIWADA.

from shortest to longest $2=4<1<3$; oval inner metacarpal tubercle at the base of the first finger and transverse and oval outer metacarpal. Subarticular tubercles small, round, formula 1, 1, 2, and 1. Toes thin, small, toe tips rounded and webbed. Small oval inner metatarsal tubercle, small rounded outer metatarsal tubercle. The dorsum is smooth with six longitudinal folds of tubercles. Several short and small rounded tubercles on the sides of the body, the dorsal surface of the tibia and the cloacal region. The venter is uniformly smooth. Dorsal surface with a pale orange non-interrupted middorsal line containing two red spots passes from the tip of the snout to the cloacal region. The loreal region, margin of lower jaw possessed dark bands. The forelimbs with light grey stripes while hindlimbs with dark stripes. The ventrum is white. The margins of the lower jaw have narrow dark bars. Males with a pair of vocal sacs and laterally dark.

Natural history notes: It is a mostly aquatic species and found in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the breeding is imitated from March or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: 170–1500 m.

Minervarya orissaensis (Dutta, 1997) (Figure 14)

Common name: Orissa Cricket Frog

Nepali name: Kithre bhyaguto

Measurement: Body size (SVL) in females 46.5–49.4 mm ($n = 2$) and 38.1–46 mm ($n = 7$) in males

Head is pointed, longer than wide, snout obtuse, canthus is obtuse; loreal slightly concave. Nares are oval and dorsally located, much closer to tip of snout than to the anterior corner of eyes. Interorbital distance is shorter than the diameter of upper eyelid (WUI = 24.5% of head length and IOD = 16% of head length). Tympanum round and almost 50% of the size of

eye. Fingers are obtusely pointed. The relative length is $2\leq 4<1<3$. Fingers without dermal fringe and are free of webbing. Subarticular tubercles are rounded. There is an oblong inner metacarpal tubercle (Thanar tubercle) at the base of the 1st finger and a transverse and oval outer metacarpal tubercle (Palmar tubercle) at the base of the other fingers. Legs are long and muscular. The relative toes length $1<5<2<3<4$, webbed toes. Inner metatarsal tubercle is shovel-shaped, long, flattened and blunt and found at the base of 1st toe equal to the length of 1st toe. Outer tubercle is small and rounded. Tibiotarsal articulation reached between tip of the snout when the hindlimb is kept parallel to the body. The dorsum is granular, brownish olive with a few dark irregular bands, mostly 2 to 3 inverted “V” shaped dark bands on the trunk. The sides of the body are marbled. A cream-colored middorsal line from the inter-orbital region to the cloaca is usually distinctive with include orange spots. The forelimbs and forelimbs are granular with regular dark bands. The ventral sides of the hand and feet are pale brown with contrasting dark brown metacarpal and metatarsal tubercles. Toe webbing is faintly marbled. The venter is uniformly white with dark bars along the margin of the lower jaw. The sides of the throat dark to black in males.

Natural history notes: It is a mostly aquatic species most found in subtropical forest, small streams, rivers waterlogged paddy fields, natural or artificial ponds, wet grassland and marshes. In Nepal, the breeding is imitated after the first monsoonal rainfall at the end of May or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Distributed in far western Nepal (below 200 m).

Genus: *Nanorana*

Molecular analyses: Three lineages of *Nanorana* have been identified from Nepal (Figure 14). The phylogenetic analysis showed *Nanorana* samples (JRK2018303–305) collected from

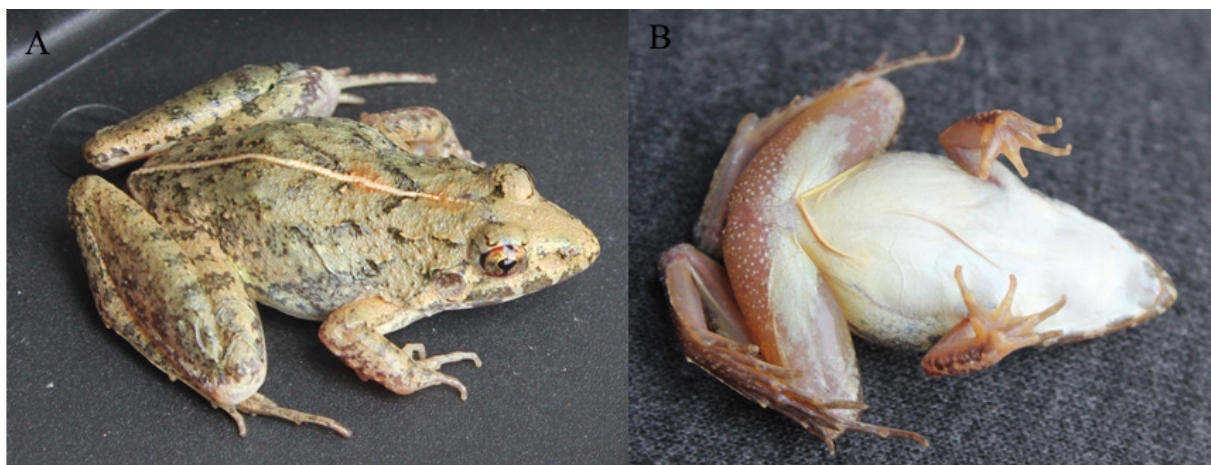


Figure 14 *Minervarya orissaensis* in life. A: dorsolateral view B: ventrolateral view. Photo by Janak Raj KHATIWADA.

Titi Lake, Mustang, Nepal, and Darchula (JRK2018161–163), Nepal showed lower genetic divergence (2.3%) with *N. blanfordii* (MH315963) from Tibet, China with lower bootstrap support value (Figure 15).

Specimen (JRK2018303, 304, 306) collected from Titi Lake, Mustang (nearby type locality of *N. rostandi*–The Kutsab Ternga Monastery, Jhomsom, Mustang, Nepal) and Pisang (JRK2017028–038), Manang, Nepal were 95%–98% similar to *N.*

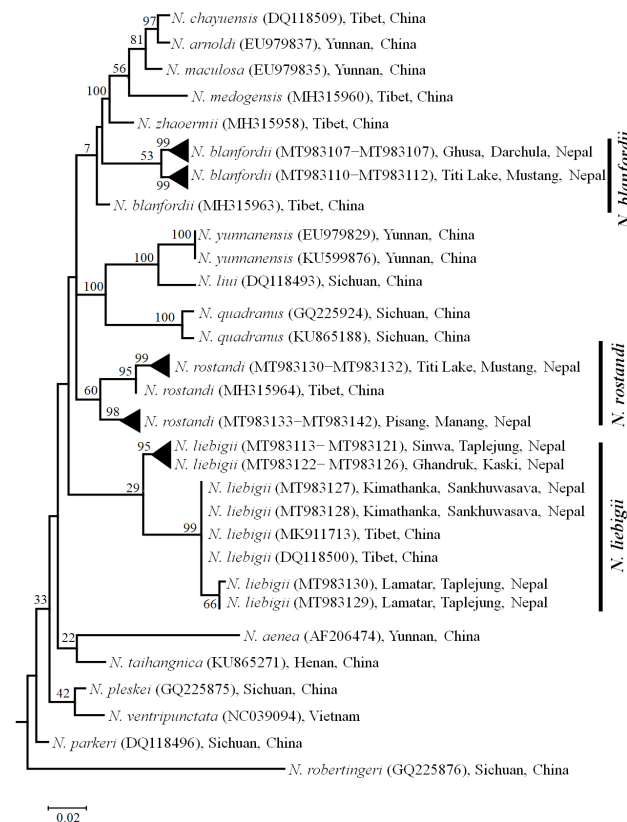


Figure 15 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the genus *Nanorana*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

rostandi (MH315964) from Tibet, China.

Nanorana samples from Ghandruk (central Nepal) and Taplejung and Sankhuwasava (eastern Nepal) were 95%–98% similar based on 16S data with the *N. liebighii* (MK911713 and DQ118500) from Tibet, China. *N. liebighii* was the sister taxon to *N. rostandi* (MH315964) from Tibet, China and *Nanorana* species from Pisang, Manang, Nepal, and the uncorrelated genetic divergence between them were 5.3%–5.6% and 2.5%–2.7% respectively.

Nanorana blanfordii (Boulenger, 1882)

Common Name: Blanford's paa frog

Nepali Name: Paha

Description: Body size (SVL) in males 65–70 mm and females 65–70 mm

The habitus is small and slender. The head wider than long. The snout is rounded in both dorsal and lateral views and juts slightly over the lower jaw. The canthus rostralis is rounded and the interorbital distance is equal to or less than the diameter of an upper eyelid. The nares are midway between the snout tip and the anterior comers of the eyes. The tympanum is indistinct and its diameter in the examined specimens was 33%–44% of the upper eyelid diameter. The supratympanic fold is weak. The pineal organ is visible as a white dot. The fingertips are pointed with a relative finger length of $1 < 2 < 3$ and only a slight difference between the 4th and 3rd fingers. The subarticular tubercles are weak and indistinct. The inner metacarpal tubercle is indistinct, rounded and lays at the base of the 1st finger. The toes are webbed with rounded tips. The subarticular tubercles are weak. The relative toe length $1 < 2 < 3 < 4$ with only one inner metatarsal tubercle which is very narrow and long, but flat. The tibio-tarsal joint reaches the nares in females, but it reaches the snout tip or beyond in males. The dorsum is smooth, with flat rounded or oblong dorsolateral warts. The dorsolateral folds lacking or feebly indicated anteriorly by the weak folds. The venter uniformly smooth. The lack of keratinized warts on the back and on the dorsal regions of the limbs. Coloration (preserved specimens): The ground colour reddish faintly marbled with a dark colour and with larger green spots on the head and back. There is a narrow faint interorbital bar and a dark stripe extends along the canthus rostralis to the temporal region. The hind limbs are marbled dorsally. The ventral side is yellowish, and the throat and anterior pectoral region are irregularly spotted and marbled. The iris is reddish with an indistinct vertical bar. Colour in live specimens: there is marked colour variation in the adult from brownish to chestnut yellow, dark or violet—with dark brown spots on greenish zones. The throat is pink and partially spotted with white. The pectoral region is yellowish with pink or brownish spots. The ventral region of the limbs is similar to the back and is banded with dark brown. The dorsum with green spots.

Reproductive males have spines on 1st and 2nd fingers and inner side of the 3rd, and on either side of the pectoral region. They have no spines on the forelimbs, but the upper and lower arm is hypertrophied. Typical female characteristics are the longer and less interrupted dorsolateral folds, a shorter and more rounded snout, and the more protruding globular eyes.

Natural history notes: It is a semiaquatic high-land species, and mostly found in the moist climate of Nepal; in the mountain streams with densely grown vegetation on the banks. In Nepal, the breeding is initiated from May to the end of July. This species is mostly nocturnal.

Distribution: Distributed in central and eastern Himalayas

(above 2500 m).

***Nanorana rostandi* (Dubois, 1974)**

Common name: Rostand's paa frog

Nepali name: Paha

Description: SVL in adult males 34.5–56.5 mm, in females 51.0–65.0 mm

Head wider than long, snout pointed in both dorsal and lateral views, slightly extended over the lower jaw, canthus rostralis round, nares closer to the anterior corners of the eyes than to tip of the snout, interorbital distance smaller than the diameter of an upper eyelid. The tympanum is relatively small, indistinct and more than its diameter from the eye with a maximum diameter of 21.4%–23.1% of the upper eyelid. A supratympanal fold is prominent. The vomerine dentition is oblique positioned and in direct contact with the choanae and posteromedial from them. The fingertips are rounded, and the relative finger length is $1 \leq 2 < 4 < 3$. The subarticular tubercles are distinctly conical. Of the three oblong oval metacarpal tubercles, the inner one is a little larger than the others. The toes are completely webbed and weakly concave. The relative toe length is $1 < 2 < 5 \leq 3 < 4$. The toe tips are rounded and slightly enlarged. The subarticular tubercles are oblong and distinct. The inner metatarsal tubercle is very long and reaches almost the length of the inner toe. The tibio-tarsal articulation reaches the nares or beyond the snout. Dorsolateral folds are interrupted at many places and formed in part by a row of oblong tubercles. The dorsal skin is smooth, with numerous rounded tubercles (some of them pale) and warts primarily in its posterior part. Those warts on the hind legs sometimes have keratinised tips. Warts are especially frequent on the knee and the dorsal surface of the lower leg. Tiny light warts are located on the side of the head below the supratympanal fold, the loreal region, and the upper eyelid. The venter is smooth with slight transverse

folds between the axilla and groin. Dorsum is grey or olive, brown to reddish and even black, with irregular brown, greenish, yellowish or reddish spots. The sides of the head, the supratympanal fold and the dorsolateral fold are dark as is the interorbital bar. The limbs have distinct dark bands. The posterior parts of the thighs are faintly violet with grey spots. The toe webbing is pale brown with greyish olive pigmentation. The venter is uniformly yellowish white to grey with a violet hue on the throat. The iris is golden divided into four zones by a horizontal and a vertical dark bar. Females larger than males and their heads become wider with age. Males have a relatively longer head and relatively long length of the lower leg. Reproductive males have nuptial fields and spines on the 1st finger (rarer on the 2nd) and sparse but large spines on the inner side of the arms. Spines on the metacarpal tubercles only develop in some specimens.

Natural history notes: It is a semi-aquatic high-land species and most found in oak and coniferous forest of Nepal; in the mountain streams with densely grown vegetation on the banks. In Nepal, the breeding is initiated from May to the end of July. This species is mostly nocturnal.

Distribution: Distributed in central and eastern Himalayas (2400–3500 m).

***Nanorana liebigii* (Gonther, 1860) (Figure 16)**

Common name: Liebig's paa frog

Nepali Name: Mana paha

Description: Body size (SVL) in females 53–104 mm ($n = 38$) and 57–136 mm ($n = 43$) in males

Head wider than long, snout blunt at lateral and projected above the lower jaw, canthus rostralis round, interorbital distance is shorter than the diameter of an upper eyelid (IOD = 21% of HL and UEW = 26% of HL), nares round and dorsolateral. The eyes are two times larger than tympanum.

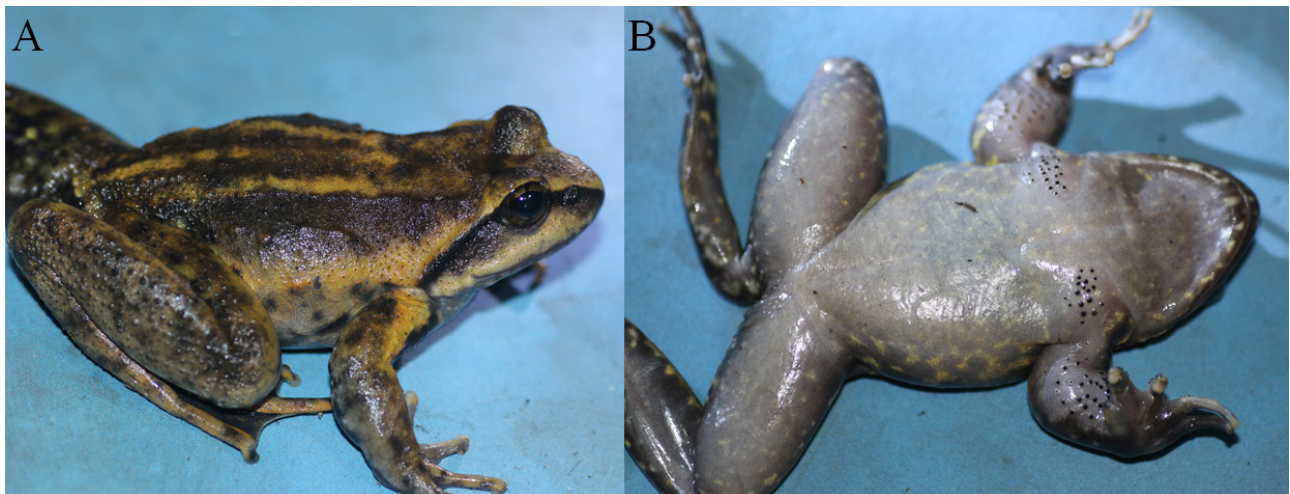


Figure 16 Male *Nanorana liebigii* in life. A: dorsolateral view; B: ventrolateral view. Photo by Janak Raj KHATIWADA.

Tympanic fold present, occipital fold located at the posterior comers of the eyes. Fingers muscular, tips rounded, and relative length $1 < 2 < 4 < 3$. Fingers without dermal fringe and are free of webbing. Single, ovoid metacarpal tubercle in the base of first finger and almost equal length of the first finger. Rounded palmar tubercle presents on the base of all three fingers. The subarticular tubercles are globular and distinct. Toes are fully webbed, and the relative toe length is $1 < 2 < 5 < 3 < 4$. The toe tips are rounded. The subarticular tubercles are oval and single an oval inner metatarsal tubercle. The tibia–tarsal articulation reaches beyond the snout. Dorsolateral folds are prominent and warty and present on either side of the flank and passes from posterior corner of eyelid up to the groin. The dorsal skin is smooth with numerous flattened and often rounded warts on the sides of the body. There are similar warts on the dorsal region of the hind limbs and in the cloacal region. The hind limbs have additional tiny white dorsal warts. There are granules on the anterior and lateral head, and in some specimens, the warts and granules have keratinised tips. The venter is uniformly smooth. The dorsal skin is very variable ranging from yellowish to greyish brown, reddish brown to chocolate or blackish brown. A dark brown stripe extends along the snout, the canthus rostralis, the supratympanal fold and the dorsolateral fold. The hind limbs with grey bands. The ventral side is uniformly white and slightly brown speckles in the gular and pectoral regions. The iris is a uniform golden-yellow with two dark lines wider horizontal and thin vertical cross each other. Usually, the males are significantly longer. Reproductive males with strongly developed nuptial spines on the pectoral region, the inner sides of the arms and on the 1st–3rd fingers, metacarpal tubercle. Some of the reproductive adult males possessed swollen circular bladder in the anal region.

Natural history notes: It is a semi-aquatic high-land species, and most found in the oak and coniferous forest of Nepal; in the mountain streams with densely grown vegetation on the banks. In Nepal, the breeding is imitated from May to the end of July. This species is mostly nocturnal.

Distribution: Widely distributed in central and eastern Himalayas (1500–3000 m).

Genus: *Sphaerotherca*

Molecular data Based on the molecular data, two lineages of *Sphaerotherca* frogs have been identified from the Nepal. *Sphaerotherca* species were suggested to form a monophyletic group based on ML analysis (Figure 17). The phylogenetic analysis showed that first lineage of *Sphaerotherca* samples from type locality of *Sphaerotherca maskeyi* Chitwan, Nepal were clustered together with *S. pashchima* **comb. nov.** (Genbank accession no: KY215993 and KY215979, type locality: Ambodi Village, Maharashtra, India). The uncorrelated genetic divergence between *Sphaerotherca maskeyi* and *S. pashchima*

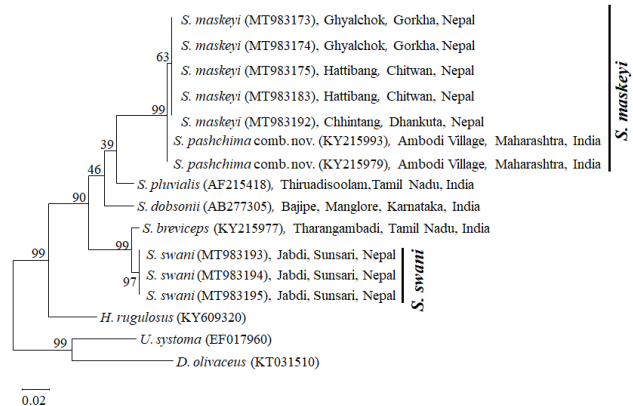


Figure 17 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the genus *Sphaerotherca*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

comb. nov. were 0.2%–0.5%. The second lineage of *Sphaerotherca* specimens from Dharan, Sunsari district, Nepal [(type locality of *S. swani* (Myers and Leviton, 1956)] were sister taxon to *S. breviceps* (KY215977–Tharangambadi, Nagapattinam District, Tamil Nadu, India) based on 16S data.

Taxonomic status of *S. pashchima* **comb. nov.**

Padhye *et al.* (2017) described a new species, *S. pashchima* **comb. nov.** (type locality: Ambodi Village, Maharashtra, India) based on molecular and photographic comparison with *S. maskeyi*. For the taxonomic studies, the photograph-based comparison might increase the potential bias in species identification and measurements (Ceriaco *et al.*, 2016). According to Padhye *et al.* (2017) *S. pashchima* **comb. nov.** has following morphological characters: first finger shorter than third, second finger equal to or less than fourth finger, distance from snout to nostril is less than half of the horizontal diameter of eye and nostrils nearer to the tip of snout, dorsum rough and warty. These morphological features are congruent with the specimens collected from Chitwan (from the holotype location of *S. maskeyi*). Our study revealed that *S. maskeyi* showed higher variability in body size and dorsal coloration (Figure 18) and as a result creates confusion in the species identification in the field. Furthermore, phylogenetic analyses revealed that *S. pashchima* **comb. nov.** (Genbank accession No: KY215993 and KY215979) found no significant genetic divergence with *S. maskeyi* (the uncorrelated genetic divergence between the *S. maskeyi* and *S. pashchima* **comb. nov.** were 0.2%–0.5%) suggesting a single biological species. Therefore, we recommend that *S. pashchima* **comb. nov.** should be considered as junior subjective synonyms of *S. maskeyi*.

***Sphaerotherca maskeyi* (Schleich and Anders, 1998)** (Figure 18)

Common name: Chitwan frog

Nepali name: Rani bhyaguto

Description: Body size (SVL) in females 51–53 mm ($n = 2$) and 41–49 mm ($n = 11$) in males

Body is short and stocky. Head is wider than long, snout short, and slightly extended over the lower jaw. The canthus is rounded and loreal region is concave. The nostrils are much closer to tip of snout than to the anterior corner of eyes. Interorbital distance is shorter than distance between nares. Interorbital distance also shorter than width of upper eyelids. The mandible with small tubercle. Tympanum distinct and round. The diameter of tympanums is equal to the distance between anterior corner of the eyes and nares. Pupils are lozenge-shaped, and iris is golden. The relative length of fingers is $2=4<3\leq 1$. First and third fingers are longer than 2nd and 4th. Fingers free of webbing and dermal fringes and with rounded tips. Subarticular tubercles are large and conical and are located at the base of fingers. Palmar and thenar tubercles are same size. The relative toes length $1<2<5<3<4$, fully webbed. Both side of 4th toe and inner margins of 5th toe possessed dermal fringes. Marginally webbed. Inner metatarsal tubercle is shovel shaped and longer than of 1st toe. Tibiotarsal articulation reached only

to the tympanum when the hindlimb is kept parallel to the body. Females are distinctly larger than males. Breeding males with a pair of sub-gular vocal sacs and nuptial pads on inner side of first finger and metacarpal tubercle. Dorsal surface is variable colour. The dorsum is smooth and reddish brown in colour from the specimens of Chitwan, Illam and Dhankuta (Figures 17A and 17B). A dark-brown triangular shaped mark on the shoulder. Reddish brown tympanum with black dots. Ventrums are creamy white. Mid-dorsal line is continuous and passes from tip of snout to the cloaca from the specimens Hattibang, Chitwan and Ghyalchok, Gorkha (Figures 17C and 17D). The dorsum is granular and reddish to yellowish brown with dark patches. There are two to three inverted V-shaped irregular dark marks. Two vertical dark bands pass from eyes to upper jaw. Hands and limbs with dark cross bars. Ventral surface of the limbs usually white in colour, throat molted with black and belly white.

Natural history notes: It is a semi-aquatic low-land species, and most found in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the

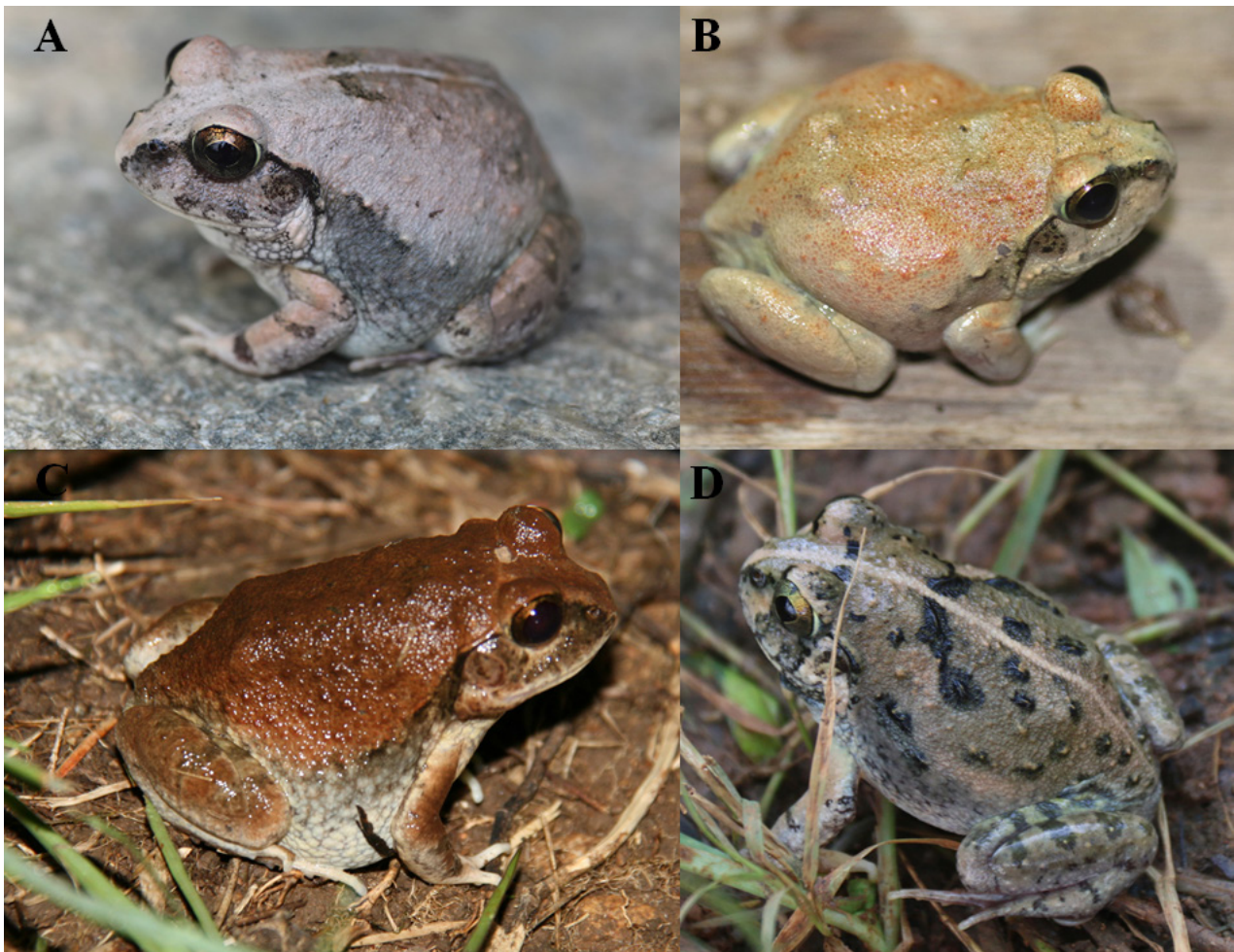


Figure 18 Different colour patterns in life of *Sphaerotheca maskeyi* collected from A: Chhintang, Dhankuta; B: Illam; C: Hattibang, Chitwan and D: Ghyalchok, Gorkha. Photo by Janak Raj KHATIWADA.

breeding is initiated after the first monsoonal rainfall at the end of May or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal. *Sphaerotheca maskeyi* is sympatric with *D. melanostictus*, *Microhyla nilphamarensis*, *Euphlyctis kalasgramensis*, and *Minervarya* sps.

General distribution: Widely distributed in Nepal (below 1200 m).

***Sphaerotheca swani* (Myers and Leviton, 1956)** (Figure 19)

Common name: Dharan bullfrog

Nepali name: Khopilte bhyaguto

Description: Body size (SVL) in females 41–42 mm ($n = 2$) and 34–37 mm ($n = 2$) in males

Body is short and stocky. Head is wider than long, snout short and blunt and slightly extended over the lower jaw. The canthus rostralis is blunt and loreal region is concave. The distance from nostrils is centre between snout and the anterior corner of eyes. Interorbital distance is shorter than width of upper eyelids ($3/4$ of upper eyelid width). Interorbital distance is shorter than distance between nares. The mandible with small tubercle. Tympanum distinct and round and about half size of eye diameter. The relative length of fingers is $1 < 3 < 2 < 4$. First and third fingers are longer than 2nd and 4th. Fingers free of webbing and dermal fringes and with rounded tips. Subarticular tubercles are large and conical and are located at the base of fingers. Palmar tubercles are distinct. The relative toes length $1 < 2 < 5 < 3 < 4$, marginally webbed. Inner metatarsal tubercle is shovel shaped and longer than of 1st toe. Tibiotarsal articulation reached only to the tympanum when the hindlimb is kept parallel to the body. Females are distinctly larger than males. Breeding males with a pair of internal vocal sacs. The dorsum is granular and yellowish brown with dark blotches. the granules are yellowish brown. Hands and limbs with dark

cross bars. Ventral surface of the limbs and belly usually white in colour.

Natural history notes: It is a semi-aquatic low-land species, and most found in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal. *Sphaerotheca swani* is sympatric with *D. melanostictus*, *Microhyla nilphamarensis*, *Euphlyctis kalasgramensis*, and *Minervarya* sps.

Distribution: Only recorded from Dharan, Sunsari district, eastern Nepal (below 250 m).

Family: Megophryidae

Genus: Megophrys

Molecular data. Based on the molecular data, three lineages of *Megophrys* frogs have been identified from the Nepal (Figure 20). The first lineage from Thiwa, Taplejung and Sulubung, Illam were 95%–100% similar with based on 16S data with the type series of *M. robusta* (KY022314) from Darjeeling, India (Mahony *et al.*, 2018). The second lineage from Raksirang and Hattibang, Chitwan district were 98%–100% similar with based on 16S data with the type series of *M. zhangi* (KX811765) from Zammu, Tibet, China (Chen *et al.*, 2017). The third lineage from Panchthar and Taplejung districts were 97%–98% similar to the *M. monticola* (KX894667) from Darjeeling district, India (Deuti *et al.*, 2017).

***Megophrys robusta* (Boulenger, 1908)**

Common name: Robust horned frog

Nepali name: Tiktike bhyaguto

For detailed morphology, please refer to Mahony *et al.* (2018) and Schleich and Kastle (2002).

This is the largest *Megophrys* frog SVL up to 114 mm with a more triangular head, superciliary ridges of the are very pronounced and look like horns (Schleich and Kastle, 2002).



Figure 19 Dorsolateral view of *Sphaerotheca swani* in life. Photo by Janak Raj KHATIWADA.

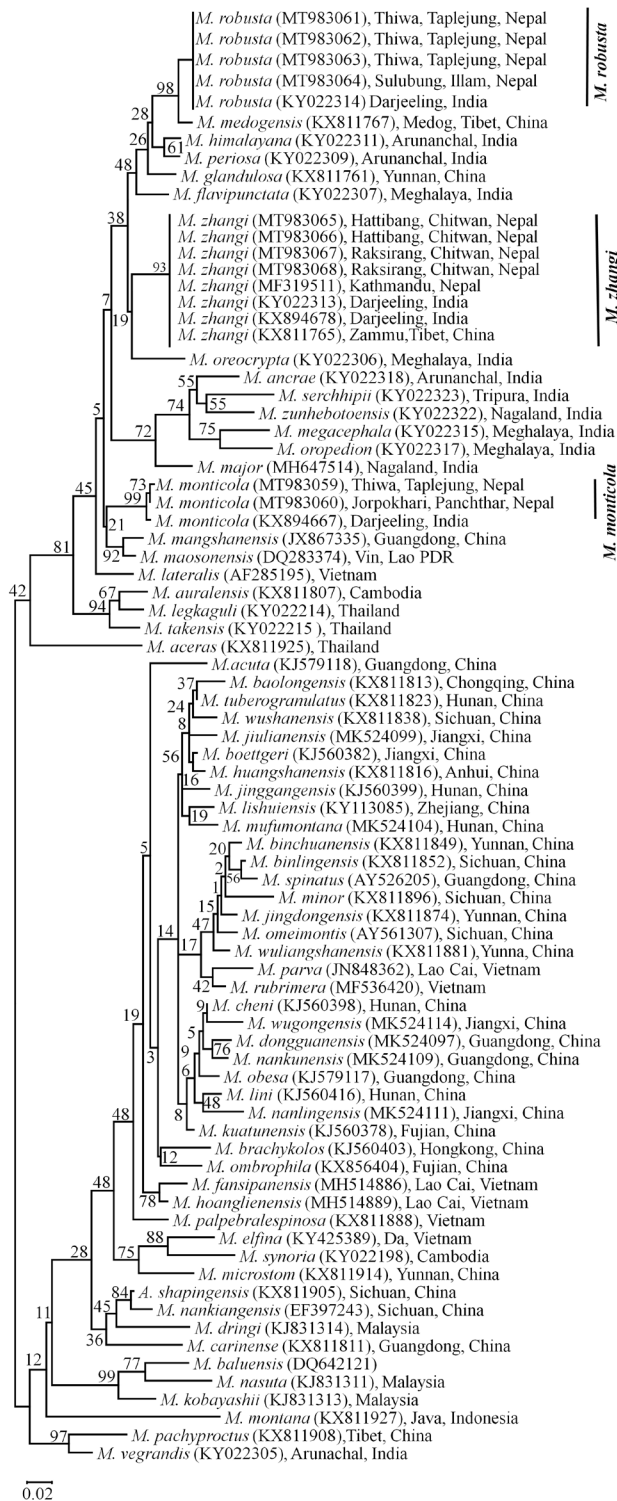


Figure 20 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the genus *Megophrys*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

Females specimen Darjeeling, India was measured up to 108 mm SVL (Mahony *et al.*, 2018).

Natural history notes: It is mostly terrestrial and found calling

from inside the bushes near the water sources (small ponds or mountain streams).

Attitudinal distribution: 1600–2400 m.

***Megophrys monticola* (Günther, 1864)**

Common name: Mountain Horned Frog

Nepali name: Tiktike bhyaguto

Description: Body size (SVL) in female 40–56 mm and male 38–48 mm.

For detailed morphology, please refer to Mahony *et al.*, (2018).

Natural history notes: It is mostly terrestrial and found calling from inside the bushes near the water sources (small ponds or mountain streams).

Attitudinal distribution: 2180–2220 m.

***Megophrys zhangi* (Ye and Fei, 1992) (Figure 21)**

Common name: Zhang's Horned Toad

Nepali name: Tiktike bhyaguto

Description: Body size (SVL) in female 37 mm and male 28–36 mm ($n = 4$).

The head is slightly flat, the head length is almost equal to width; snout round and slightly protruding from the lower jaw, tympanum small and round; vomerine teeth poorly developed, maxillary teeth present, canthus rostralis distinct, nares laterally placed. The forelimbs are thin, total length of arms and are less than half the SVL. The relative length of fingers is $1=2<4<3$. Fingers free of webbing and dermal fringes and with rounded tips. Subarticular and metacarpal tubercles absent; thenar tubercle weakly developed. The hind limbs are relatively long and thin, toes thin without lateral fringes, relative toes length $1<2<5<3<4$, marginally webbed, outer metatarsal, subarticular and supernumerary tubercles absent, inner metatarsal tubercle poorly developed. Tibiotarsal articulation reached to the anterior corner of eye when the hindlimb is kept parallel to the body.

Skin on dorsal surfaces of snout, head smooth, back weakly granular; throat, chest, and limbs smooth; flanks moderately granular, posterior thighs and cloacal region finely granular. Flanks, and dorsal surface of head, body, and limbs primarily golden brown, faint solid dark brown triangular marking on dorsal surface of head; slightly oblique dark bar below each eye; tip of snout brown, gular and chest region brown with some dark blotches; abdomen white with irregular brown blotches, brown bars present on dorsal surface of limbs and fingers, ventral surfaces of thighs and shanks primarily orange with small white dots. Females are distinctly larger than males. Breeding males with a pair of external vocal sacs.

Natural history notes: Most males call during May and June, along the banks of small temporary and permanent streams bordered by dense low vegetation. They inhabit in small streams from moderately disturbed secondary growth to mature

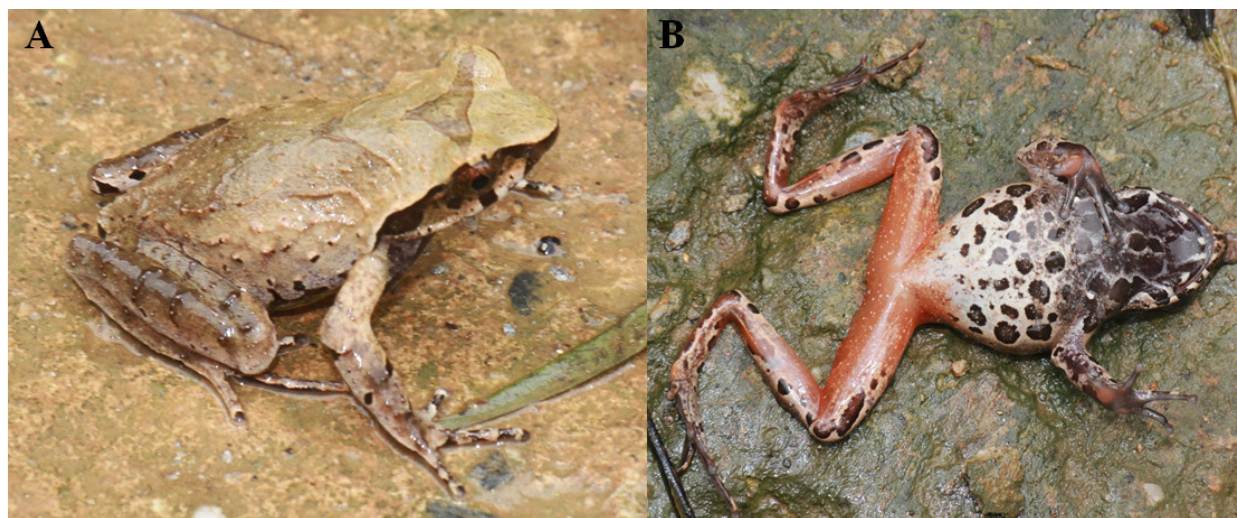


Figure 21 *Megophrys zhang* in life A: dorsolateral view, and B: ventral view. Photo by Janak Raj KHATIWADA.

primary growth forest.

Distribution: Widely distributed in central and eastern Nepal (700 and 1000 m)

Genus: *Scutiger*

Molecular analysis

Four distinct species of *Scutiger* frogs: *S. ghunsa*, *S. sikkimensis*, *S. Boulengeri*, and *S. nepalensis* have been revealed by the molecular analysis based on 16S sequences (Figure 22). For further details see in the (Khatiwada *et al.*, 2019a).

Scutigera boulengeri (**Bedriaga, 1898**) (Figure 23)

Common names: Boulenger's high altitude toad, Boulenger's lazy toad, Xizang Alpine Toad

Nepali name: Lekhali khasre

Measurements: Body size (SVL) in females 41–62 mm (Nanhoe and Ouboter 1987) and 33–38 mm ($n = 2$) in males.

Head is flattened, wider than long, canthus rostralis distinct and rounded, supratympanal fold very narrow and thin, tympanum indistinct, parotoids oblong located in between the posterior corners of the eye to arm insertion, eye size medium, pupil vertical, width of upper eyelid is equal to interorbital distance, nares are lateral placed. Arm robust, short (FAL:SVL 24%) and shorter than hand (HAL:SVL 50%); relative finger lengths I<II<IV<III; fingers tip round, not dilated; fingers without distinct lateral fringes, webbing absent; subarticular tubercles indistinct; metacarpal tubercles indistinct; nuptial spines on dorsal and lateral surface of first and second fingers, but only on inner side of third finger. Hindlimbs powerful and long, tibiotarsal articulation reaches posterior corner of eyes when hindlimb pressed parallel to body; toes thin and long, relative length I<II<III<V<IV; toe tips round; webbing absent,

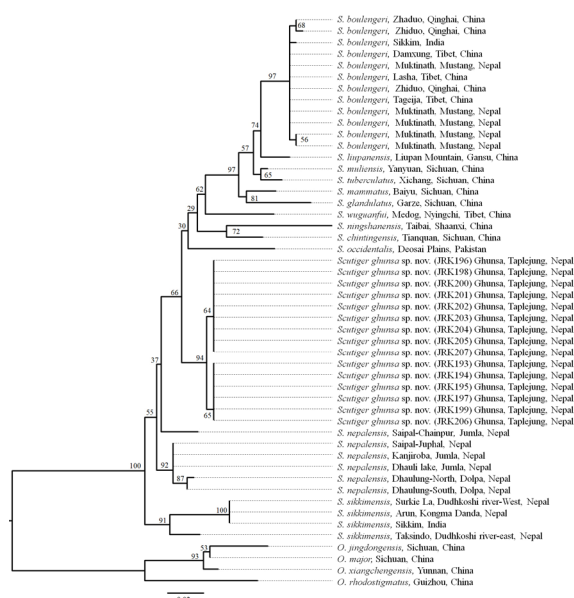


Figure 22 Maximum likelihood tree based on 16s gene sequences adopted from Khatiwada *et al.* (2019a).

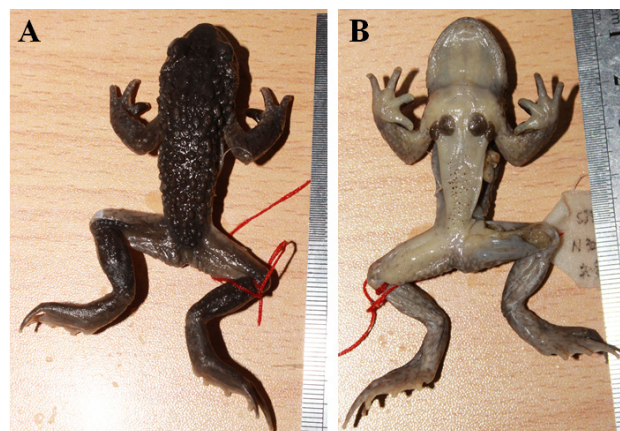


Figure 23 Male *Scutigler boulengeri* in preservative A: dorsal view; B: ventral view. Photo by Janak Raj KHATIWADA.

large and oblong inner metatarsal tubercle. Dorsal skin smooth, few small porous warts arranged on the longitudinal rows, larger warts in the cloacal region. Ventrums smooth, a pair of pectoral and axillary glands present on chest, pectoral glands two times longer than axillary glands, glands covered by minute, dense black spines. Light greenish grey or olive, dorsal with somewhat rounded black blotches with white dot in the centre, limbs with irregular dark bands, ventrum is lighter and uniformly yellowish to yellowish grey.

Natural history notes: They are found in a mixed sub-alpine forest with low canopy cover with a slow running stream and stagnant water pools.

Distribution: Widely distributed in central and eastern Himalayas (3200–5300 m).

***Scutiger nepalensis* (Dubois, 1974)**

Common name: Nepal high altitude toad

Local name: Nepali lekhali khasre

Description: Body size (SVL) in females 60.4–66.8 mm ($n = 2$) and 69.7–76.0 mm ($n = 4$) in males (Khatiwada *et al.*, 2019a).

For detailed morphology, please refer to (Dubois 1974b).

Natural history notes: They are found in a mixed sub-alpine forest with low canopy cover with a slow running stream and stagnant water pools.

Distribution: 2920–3100 m in western Nepal

***Scutiger sikimensis* (Blyth, 1854)**

Common name: Sikkim high altitude toad

Local name: Sikkime lekhali khasre

Description: Body size (SVL) in males 44.0–61.5 mm, females 47.0–60.5 mm (Nanhoe and Ouboter 1987).

For detailed morphology, please refer to (Dubois 1974).

Natural history notes: They are found in a mixed sub-alpine forest with low canopy cover with a slow running stream and stagnant water pools.

Distribution: Distributed in the eastern Himalayas (2800–4116 m).

Scutiger ghunsa (Khatiwada, Shu, Subedi, Wang, Ohler, Canatella, Xie, Jiang 2019) (Figure 24)

Local Name: Ghunsa alpine toad

Nepali Name: Ghunsa khasre bhyaguto

Description: Body size (SVL) in males 42.1–47.7 mm, females 50.2–53.9 mm

Moderate body size; head wider than long; snout short and round large paratoid glands on either side of head between posterior corner of eye to armpit (axills); canthus rostralis distinct; nostril dorsolateral, just below canthal ridge; loreal region slightly concave, eyes large and convex; eye diameter almost similar to snout length; tympanum and supratympanic fold absent; interorbital space flat, interorbital distance (greater than width of the upper eyelid; internarial surface flat; choanae small; vomerine teeth and ridges absent; maxillary teeth absent; tongue oval, deeply notched posteriorly, lingual papillae absent; openings of vocal sacs absent. Arm robust, short (and shorter than hand; relative finger lengths $I < II < IV < III$; third finger shorter than arm; fingers tip round, not dilated; fingers without distinct lateral fringes, webbing absent; subarticular tubercles indistinct; flat, large and ovoid metacarpal tubercles, inner tubercle almost equal in size to outer tubercle; nuptial spines on dorsal and lateral surface of first and second fingers, but only on inner side of third finger. Hindlimbs powerful and long, tibiotarsal articulation reaches tympanum when hindlimb pressed parallel to body; shank almost equal to thigh but tarsus short, foot length longer than shank and thigh; toes thin and long, relative length $I < II < III < V < IV$ (fourth toe of right foot cut for molecular analysis); toe tips round; weakly webbed; subarticular tubercles indistinct; inner metatarsal tubercle flat and oval; outer metatarsal tubercle poorly developed;

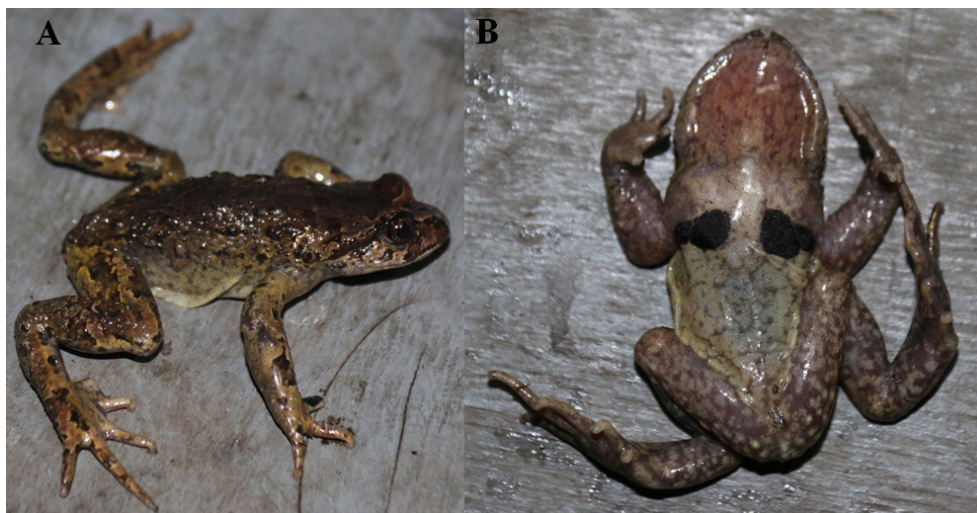


Figure 24 Male *Scutiger ghunsa* in life A: dorsolateral view; B: ventral view. Photo by Janak Raj KHATIWADA.

supernumerary and plantar tubercles absent. Skin of dorsum rough with scattered warts bearing black spines, density of warts and granules increasing towards vent; dorsal surface of snout and interorbital region smooth; few scattered tubercles on upper and lower mandibles; dorsal surface of forelimbs and hindlimbs with smaller tubercles than back; flanks with larger white warts or granules; side of shank with white warts or glandular tubercles without spines; a pair of pectoral and axillary glands present on chest, pectoral glands two times longer than axillary glands, glands covered by minute, dense black spines; vent surrounded by granules. An olive brown triangular spot on snout and interorbital region; posterior surface of head and dorsal body light brown; alternating olive brown and dark brown bands on upper lip; iris golden; forelimbs and hind limbs olive brown; irregular black spots on both limbs including fingers and toes; flanks light brown and gradually fading into creamy yellow ventrally. Ventral surface of head light brown; chest and abdomen creamy white with irregular light gray-brown lines; ventral surface of limbs brown with small irregular creamy white spots. The females are larger in SVL than males. Males with nuptial spines on the dorsal surface of first three fingers (absent in females); a pair of pectoral and axillary glands present on chest (absent in females). Females with smooth skin, body surface devoid of spines; warts flatter on dorsal body surface than in males.

Natural history notes: They were found in a mixed rhododendron forest with low canopy cover with a slow running stream and stagnant water pools.

Distribution: Only recorded from Ghunsa, Taplejung, Nepal (3500 m).

Family: Microhylidae

Genus: *Microhyla*

Molecular analyses: Two distinct species of *Microhyla*: *M. taraiensis* and *M. nilphamariensis* have been revealed from Nepal by the molecular analysis based on 16S sequences (Khatiwada *et al.*, 2017). For further details see the Khatiwada *et al.* (2017) (Figure 25).

Microhyla taraiensis (Khatiwada, Shu, Wang, Thapa, Wang, and Jiang, 2017) (Figure 26)

Local Name: Tarai narrow mouth frog

Nepali Name: Tarai kir kire bhyaguto

Description: Head relatively broad, snout truncate, eyes not protuberant and not visible when viewed from the ventral side. Canthus rostralis indistinct, nostril closer to the tip of the snout than to the eye, tympanum hidden, supratympanic fold indistinct, moderate eye size, inter-orbital distance is greater than the inter-narial distance, elliptical tongue, maxillary and vomerine teeth absent. LA shorter than LH, fingers thin, free of webbing, fingertips round and not dilated, relative length of fingers $1 < 2 < 4 < 3$. Rounded inner metacarpal tubercle two times

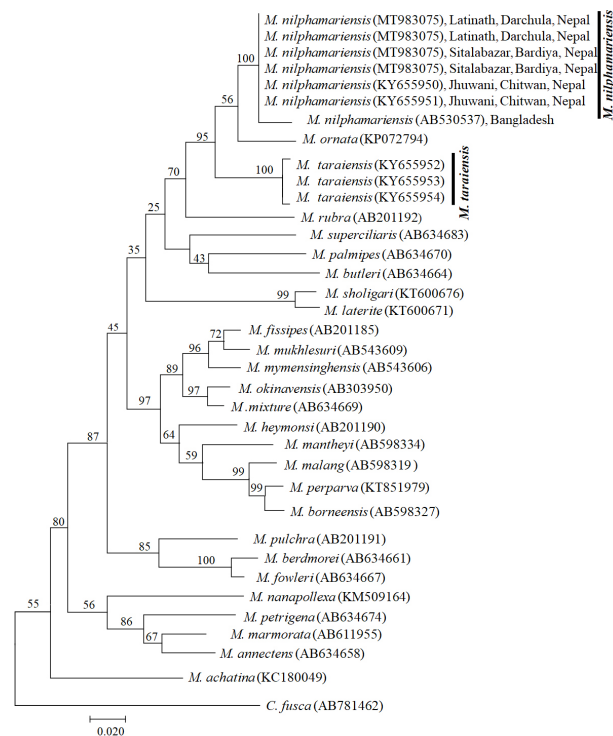


Figure 25 Maximum likelihood tree based on 16S gene sequences adopted from Khatiwada *et al.* (2017).

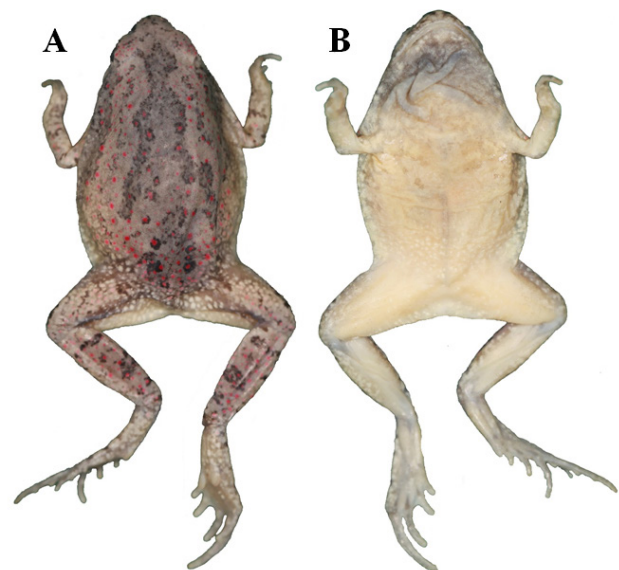


Figure 26 *Microhyla taraiensis* in preservative. A: dorsolateral view; B: ventral view. Photo by Janak Raj KHATIWADA.

greater than outer metacarpal; nuptial pads absent. Subarticular tubercles small, round, formula 1, 2, 3, and 2. Tibiotarsal articulation reached the nostril when the hindlimb is kept parallel to the body. Hindlimbs muscular and slender; toes thin and small, toe tips rounded, relative length of toes $1 < 2 < 5 < 3 < 4$, webbing weakly developed. Elongated and bean-shaped inner metatarsal measuring almost half the length of the first toe.

Rounded outer metatarsal tubercle. All five toes had small and round subarticular tubercles, formula 1, 1, 2, 3, and 2. Skin light brown with small red spots present all over the dorsal surface of the body, forelimbs and hindlimbs, except on the hand, metatarsus and foot. A rectangular black marking was present in the interorbital region; two long black stripes extended from the orbital region to the groin. Irregular dark bands are present: one band on the arm, two on the hand, one on the femur and three on the tibia. Pupil black; iris golden yellow. Belly creamy white; throat brown in the female and blackish grey in males. The females are larger in SVL than males. Males with nuptial spines on the dorsal surface of first three fingers (absent in females); a pair of pectoral and axillary glands present on chest (absent in females). Females with smooth skin, body surface devoid of spines; warts flatter on dorsal body surface than in males.

Natural history notes: *M. taraiensis* is a semi-aquatic low land species, and only recorded from Jamun Khadi, Jhapa district Nepal. It inhabits in the sub-tropical low land in humid habitats in waterlogged paddy fields, natural or artificial ponds, wet grassland and marshes. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Only recorded from Jamuna Khadi, Jhapa (150 m).

Microhyla nilphamariensis (Howlader, Nair, Gopalan, and Merilä, 2015) (Figure 27)

Local Name: Narrow mouth frog

Nepali name: Kirkire bhyaguto

Head is largely than wider in males while head is more or less squared shaped in female. Nostril closer to eyes than to tip of snout, interorbital distance larger than width of upper eyelid, inter-orbital distance is greater than the inter-narial distance, elliptical tongue, maxillary and vomerine teeth absent, hidden



Figure 27 Dorsolateral view of *Microhyla nilphamariensis* in life. Photo by Janak Raj KHATIWADA.

tympanum, supratympanic fold indistinct and vomerine teeth absent. Arm shorter than hand, thin and small fingers with free of webbing, round tips, relative length of fingers from shortest to longest $1 < 2 < 4 < 3$. Hindlimbs much muscular and slender than fore arms; Toes are free of webbing, rounded tips and relative length of toes from shortest to longest $1 < 2 < 5 < 3 < 4$. Some of the Nepalese specimens with small orange dots on the back. Dorsum light brown with distinct dark brown diamond-shaped marking on the back. The limbs with dark cross bars. The belly white; the throat and chest are brown.

Natural history notes: *M. nilphamariensis* is a semi-aquatic low-land species and wide distribution in Nepal from east to west. It inhabits in the sub-tropical forest, waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Widely distributed in Nepal (70–1700 m).

Genus: *Uperodon*

Molecular analyses: The phylogenetic analysis based on 16S gene identified the two species of microhylid genus *Uperodon* (Figure 28). *Uperodon* samples from eastern Nepal were 91% similar based on 16S data with the type series of *U. taprobanicus* (MG557966), from Karnataka, India (Garg *et al.*, 2018) and the uncorrelated genetic divergence between them were 0.9%. *U. taprobanicus* was the sister taxon to *U. conjuncta* (AY326064) from Philippines (KM247362) and *U. pulchra* (KC822614), China and the uncorrelated genetic divergence between them was 8.7% and 9.2% respectively. Based on the phylogenetic analysis a single specimen from Prakashpur, Sunsari, Nepal (showed low

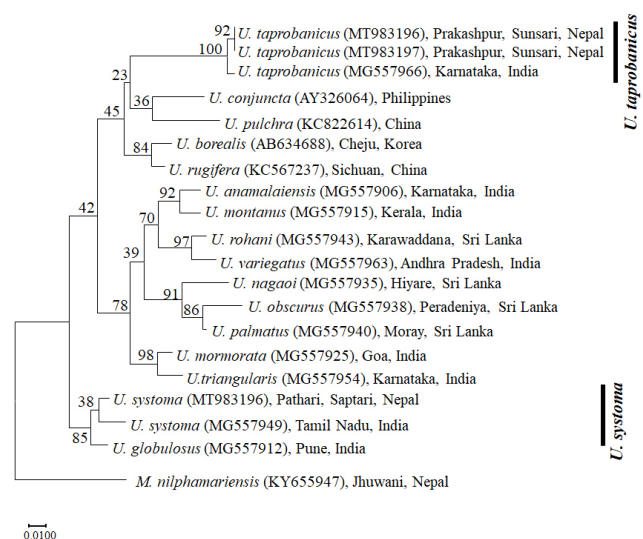


Figure 28 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the species of the genus *Uperodon*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

bootstrap support value) was genetically similar with *U. systoma* (MG557949), Tamil Nadu, India (Garg *et al.*, 2018). However, the uncorrelated genetic divergence between them were 2.2%.

***Uperodon taprobanica* (Parker, 1934) (Figure 29)**

Common Name: Sri Lanka bullfrog

Nepali name: Pangre bhyaguto

Description: Body size (SVL) in males 41.5–56.2 mm ($n = 3$) and 44.5–52.5 mm ($n = 14$) in females (Schleich and Kästle, 2002).

Body globular; head is wider than long (almost double size of length), snout very short and blunt, interorbital distance is wider than the width of an upper eyelid, canthus rostralis



Figure 29 Dorsolateral view of *Uperodon taprobanica* in life. Photo by Janak Raj KHATIWADA.

rounded; loreal region obtuse; tympanum indistinct. Arms short and strong, shorter than hand; finger free of webbing and tips with truncate discs; relative finger length $1 < 2 < 4 < 3$; subarticular tubercles small and rounded; formula for subarticular tubercle 1, 1, 2, 2; inner metacarpal tubercle oval and small and lies at the base of 1st finger and outer one bilobed. Hind limbs short and strong; length of thigh shorter than shank and foot; toes

tips rounded, partially webbed (about one third of their length); dermal fringes weakly developed; a pairs metatarsal tubercle is shovel-shaped; inner tubercles one and half times larger than the outer one. The tibio-tarsal articulation reaches to the shoulder when hind limb kept parallel to the body. Dorsum smooth with scattered glandular tubercles. Dorsal surface of head and body dark brown; a reddish-orange stripe in between eyes; a pair of reddish-orange or brick-red stripes passes from the posterior corner of eyes to the abdomen laterally; lateral surfaces of head light reddish-orange, and flanks light brown; forelimbs, dorsal surfaces of thigh, shank and foot, light brown with pale reddish-orange; fingers and toes light brown with grey spots. Males with a large external gular vocal sac.

Natural history notes: It is a semi-aquatic low-land species, and most found in the sub-tropical low land areas of Nepal in humid habitats in waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the breeding is imitated after the first monsoonal rainfall at the end of May or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal. *U. taprobanicus* spend the whole dry period dug into the soil and also recorded from the tree trunk and logs near the water sources.

Distribution: Widely distributed in low land Nepal (below 400 m).

***Uperodon systoma* (Schneider, 1799) (Figure 30)**

Common name: Marbled balloon frog

Nepali name: Matyangre ghamndi bhyaguto

Description: Body size (SVL) in male 40.2 mm ($n = 1$) from Pathri, Saptari district and SVL of 47.5–49.5 mm for males ($n = 6$) and 48.5–52.5 ($n = 2$) in females (Schleich and Kästle 2002).

Stocky habit, head small and short; distinctly wider than long; snout rounded, slightly projecting over the lower jaw; centrally, canthus rostralis indistinct and blunt. The interorbital

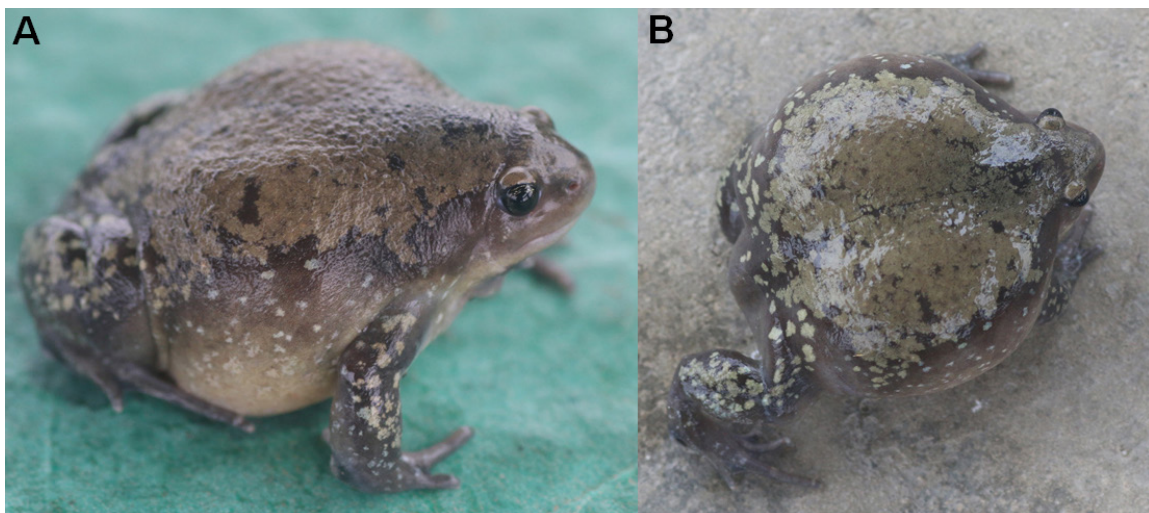


Figure 30 *Uperodon systoma* in life. A: dorsolateral view; B: aggressive posture. Photo by Janak Raj KHATIWADA.

distance almost two times of the width of an upper eyelid. Pupil rounded to slightly lozenge shaped, tympanum hidden. Short and muscular forelimbs, fingertips rounded; relative finger length $1 < 4 < 2 < 3$; with a rounded inner and a drop-shaped outer metacarpal tubercle and rounded subarticular tubercles. Hindlimbs relatively shorter, toe length $1 < 5 < 2 < 3 < 4$; subarticular tubercles not prominent; toes partially webbed; two large metatarsal tubercles are flattened and shovel-shaped, the inner one of about double length; the tibiotarsal articulation does not reach to the axilla. Dorsum almost smooth with few tubercles, slightly or strongly tubercular; venter generally smooth. Dorsum and dorsal side of limbs intensely marbled with yellow and dark brown; venter creamy white to yellowish; throat dotted with dark brown in males. Males with subgular vocal sac.

Natural history notes: It is a semi-aquatic low-land species, and most found in the sub-tropical low land areas around Sal forest (*Shorea robusta*) and margins of agricultural fields, waterlogged paddy fields, natural or artificial ponds, wet grassland, and marshes. In Nepal, the breeding is imitated after the first monsoonal rainfall at the end of May or early June. It breeds into shallow water either standing or running, rice fields and ditches. This species is mostly nocturnal.

Distribution: Distributed in low land Nepal (below 200 m).

Family: Ranidae

Genus: *Hydrophylax*

Molecular data. The phylogenetic analysis showed that *Hydrophylax* samples from Nepal were closely nested with *Hydrophylax leptoglossa* based on 16S data (Figure 31). Nepalese samples were 99%–100% similar to the type series of *H. leptoglossa* (AB530528), from Kyaukpyu, Myanmar (Oliver et al., 2015) and *H. leptoglossa* (KM069011), Manipur, India (Biju et al., 2014). *H. leptoglossa* was the sister taxon to *H. krefftii* (KM247362) from Solomon Islands Madikeri and *H. malabarica* (KM068966) from Meladoor, Western Ghats, India and the uncorrelated genetic divergence between them was 3.4 and 2.9% respectively.

***Hydrophylax leptoglossa* (Cope, 1868)** (Figure 32)

Common name: Cope's Assam frog

Nepali name: Bhyaguto

Description: Body size (SVL) in females 41–62 mm ($n = 8$) and 30–45 mm ($n = 20$) in males

Head broader than wide, snout pointed, canthus rostralis indistinct, nostrils much closer to tip of snout than to eyes. Interorbital distance broader than the diameter of upper eyelid. Tympanum round and almost half of the size of eye. The relative length of fingers is $1 = 2 < 4 < 3$. Fingers are free of webbing. Single and rounded subarticular. Fingers devoid of dermal ridge. Toes and fingertips are rounds. The relative

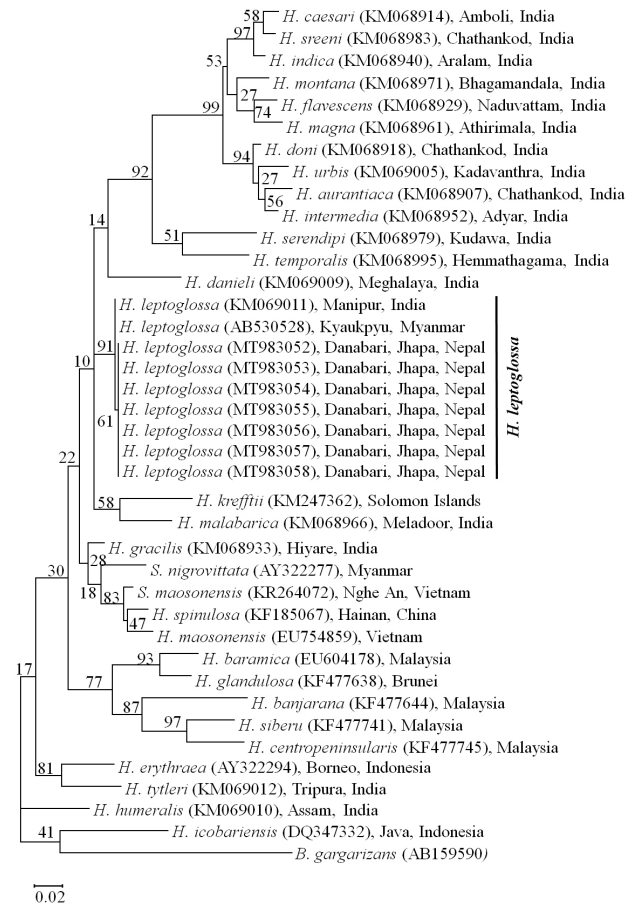


Figure 31 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the species of the genus *Hydrophylax*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

toes length $1 < 2 < 5 < 3 < 4$, fully webbed, two oval meta-tarsal tubercles, elongated and compressed inner metatarsal tubercle. Oval and small outer metatarsal tubercle. Dorsal surface of the body is greyish brown with darker rounded spots. Limbs with incomplete dark bands. Ventral surface is white.

Natural history notes: All individuals were observed in temporary or permanent pools, around sal forest and agricultural field. This species is mostly nocturnal. In Nepal, the breeding is initiated from March to August. It breeds into shallow water either standing or running, rice fields and ditches. Females are much larger than males.

Distribution: Widely distributed in low land Nepal (below 400 m).

***Amolops* (Cope, 1865)**

Based on the molecular data, three lineages of *Amolops* frogs have been identified from Nepal (Figure 33). The phylogenetic analysis showed that first lineage of *Amolops* samples from (Barahakshetra, Sunsari; Hattibang, Chitwan; Pokhara, Kaski; Latinath, Darchula) and were identified as *Amolops*

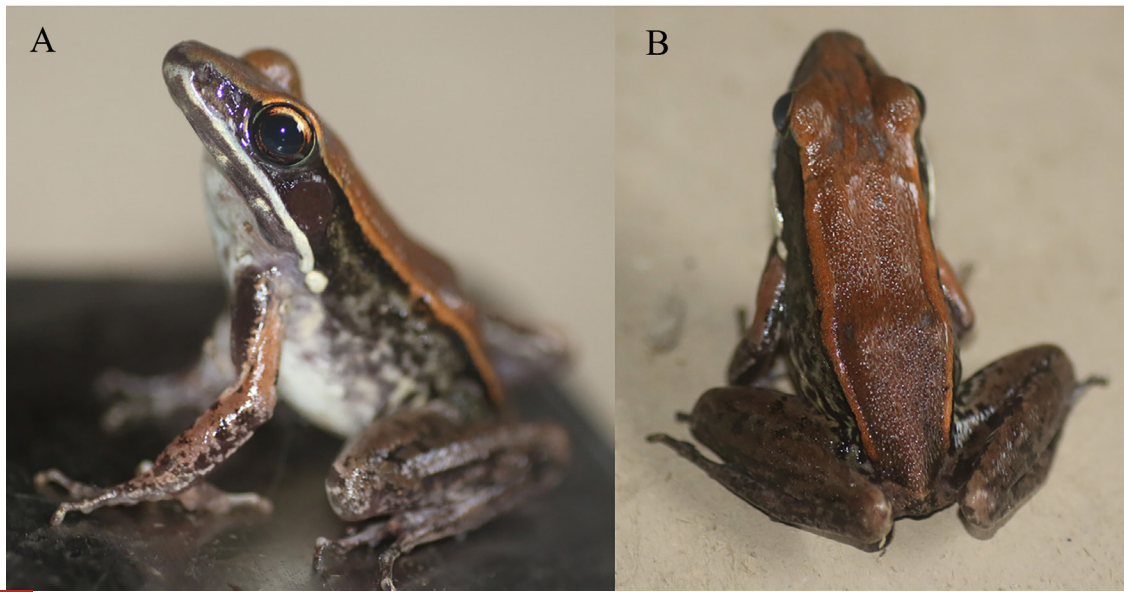


Figure 32 *Hydrophylax leptoglossa* in life A: dorsolateral view; B: dorsal view. Photo by Janak Raj KHATIWADA.

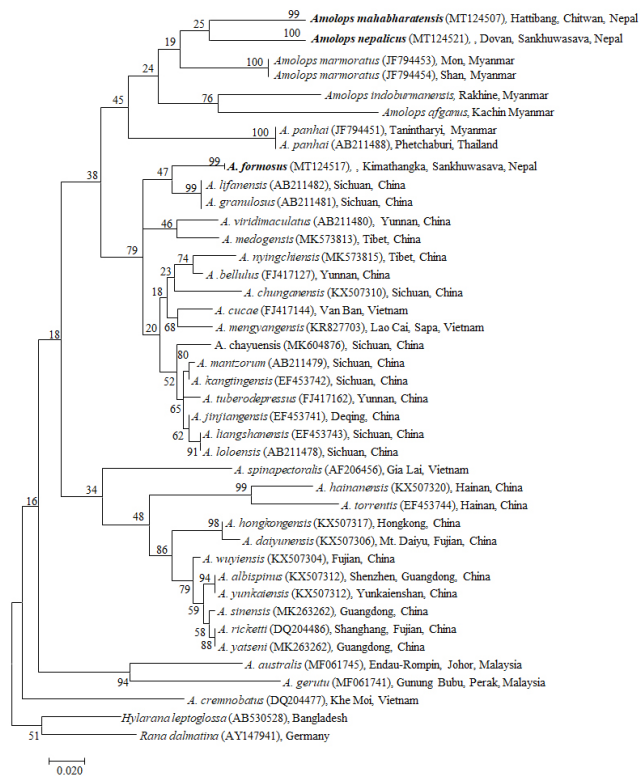


Figure 33 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the species of the genus *Amolops*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

mahabharatensis. The second lineage from Lamatar, Taplejung and Kimathanka, Sankhuwasava were identified as *Amolops formosus*. The third lineage from Dovan, Sankhuwasava was identified as *Amolops nepalicus*. The uncorrelated genetic

divergence between *A. mahabharatensis* and *A. nepalicus* was 13%, with topotypic *A. marmoratus* was 12% and *A. panhai* was 15%. Similarly, *A. nepalicus* was sister taxon of *A. panhai* and the uncorrelated genetic divergence between them was 15% whereas with *A. marmoratus* was 13%. The genetic distance between *A. formosus* with *A. mahabharatensis* and *A. nepalicus* were 13% and 15% respectively.

***Amolops mahabharatensis* (Khatiwada, Shu, Wang, Zhao, Xie, Jiang 2020) (Figure 34)**

Common name: Mahabharat torrent frog

Nepali name: Pahadi pirre paha

Description: Body size (SVL) in females 41–62 mm ($n = 8$) and 30–45 mm ($n = 20$) in males

Head wider than long and flat; snout short and round; canthus rostralis distinct; canthus slightly projected towards snout; nostril lateral, just below canthal; loreal region slightly concave, small eyes and convex; eyes diameter shorter than snout length; tympanum small; interorbital distance, greater than width of the upper eyelid, and internarial distance; supratympanic fold present, extended from back of eye

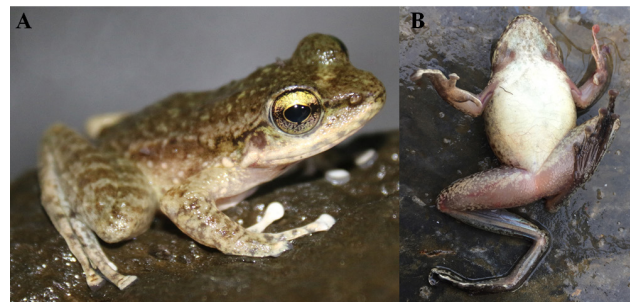


Figure 34 *Amolops mahabharatensis* in life A: dorsolateral view; B: ventral view. Photo by Janak Raj KHATIWADA.

to shoulder; small spine present between the junction of supratympanic fold and upper mandible; paratympanic fold absent; choanae moderate; vomerine teeth weakly developed; tongue lanceolate; deeply notched posterior; gular poaches present. Arm robust and shorter than hand; relative fingers lengths I<II<IV<III; third finger shorter than arm; fingers tip dilated with oval disks with circummarginal grooves; third disk width, greater than tympanum; nuptial pad in first finger, without conical spines, fingers without distinct lateral fringes, webbing absent; rounded subarticular tubercle; formula for subarticular tubercles: 1, 1, 2, 1; outer metacarpal tubercle ovoid, flat and larger than inner tubercle, round and small. Hindlimbs powerful and long, tibiotarsal articulation reaches the snout when hindlimb is kept parallel to the body; shank longer than thigh, tarsus; toes thin and long, relative lengths I<II<III<V<IV; toes tips with semicircular disks with semicircular grooves; fully webbed; subarticular tubercles slightly ovoid; formula for subarticular tubercles: 1, 1, 2, 3, 2; inner metatarsal tubercle prominent and oval; outer metatarsal poorly developed; supernumerary and plantar tubercles absent. Skin granular in dorsal head, body, limbs, fingers, toes and flank region. The dorsum is greyish olive; dorsolateral and supratympanic folds light brown; ventral white; iris pale yellow to light golden, pupil black. Females larger than males.

Natural history notes: All individuals were observed in the torrent habitat with high canopy cover. The vegetation is subtropical mixed forest. Adults of *A. mabharatensis* were collected from rock in the fast-flowing stream with more than 90% of rock cover. In Nepal, the breeding is initiated from May to September. Females are much larger than males.

Distribution: Widely distributed in Nepal (700–1400 m).

Amolops nepalicus (Yang, 1991) (Figure 35)

Common name: Nepal torrent frog

Nepali name: Pirre Paha

Description: Body size (SVL) in females 41–62 mm ($n = 8$) and 30–45 mm ($n = 20$) in males

Head relatively wide; canthus rostralis distinct; eye moderate; pupil round; tympanum small and round; inter-orbital distance is greater than the inter-narial distance; snout flat and round and is shorter than nostril to eye distance; width of upper eyelid almost equal length to the interorbital width. Vomerine teeth presence between choanae. Dorsolateral folds indistinct. Arm shorter than length of hand; fingers slender, free of webbing and tips with large and wide disc with circummarginal groove which are larger than those of the toes; relative length of fingers from shortest to longest $F1 \leq F2 < F4 < F3$; subarticular tubercles distinct and bulging; supernumerary tubercles small and round, located at bases of all fingers. Hindlimbs moderately slender; tibiotarsal articulation reached the snout when the hindlimb is kept parallel to the body. Toes strong and long with large disc those of third and fourth digits, relative length of toes $T1 < T2 < T5 < T3 < T4$, toes fully webbed, subarticular tubercles small, round and distinct; metatarsal tubercle indistinct. Skin with few rounded tubercles scattered on back; dorsolateral fold indistinct; small tubercles on outer side of thigh; belly smooth. Light brown or white on back with dark brown spots on tubercles in alcohol; darker brown and black crossbars on limbs; belly yellowish grey. Male with paired gular pouches.

Ecological notes: *A. nepalicus* inhabits in the torrent habitat with high canopy cover. The vegetation is subtropical mixed forest. Adults were collected from rock in the fast-flowing stream with more than 90% of rock cover.

Distribution: This species is known with certainty only from the Sabha Khola and Dovan, Sankhuwasabha district, Nepal (900–1400 m).

Amolops formosus (Guenther, 1875) (Figure 36)

Common name: Assam torrent frog

Nepali name: Lekali pirre paha

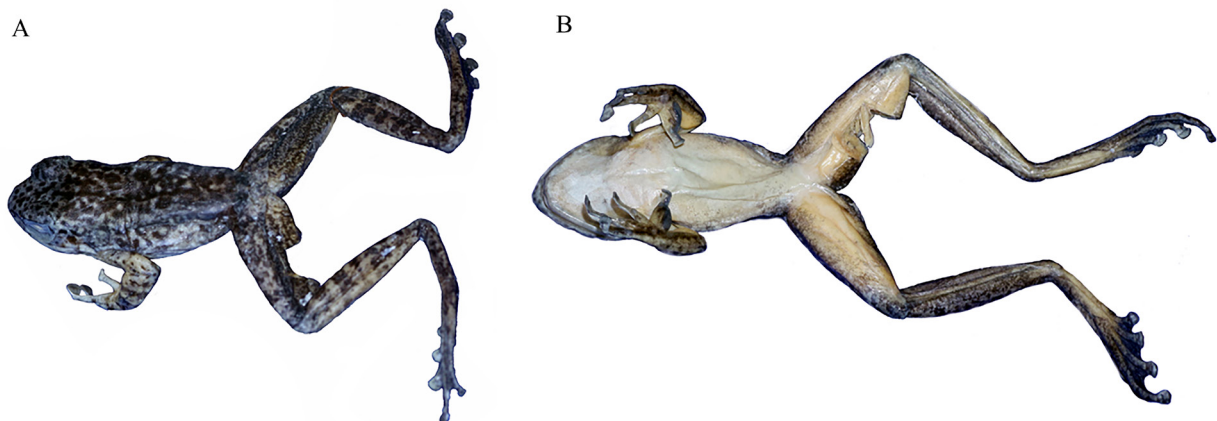


Figure 35 *Amolops nepalicus* in life. A: dorsolateral view; B: ventral view. Photo by Janak Raj KHATIWADA.

Head relatively wider; canthus rostralis distinct; eye of moderate; pupil round; tympanum small and round; inter-orbital distance is greater than the inter-narial distance; snout flat and rounded, snout longer than nostril to eye distance, width of upper eyelid almost half-length to the interorbital width. Presence of small granules at angle of jaws and extra tympanic area. Dorsolateral folds indistinct. Arm shorter than hand; fingers muscular, free of webbing, fingertips with large and wide disc with circummarginal groove and larger than those of the toes; relative length of fingers from shortest to longest $F1 < F2 < F4 < F3$; subarticular tubercles distinct and oval. Elongated inner metacarpal tubercle, smaller than outer metacarpal. Hindlimbs muscular and slender. Toes strong and long with large disc those of third and fourth digits; relative length of toes $T1 < T2 < T5 < T3 < T4$; toes fully webbed, subarticular tubercles oval and distinct; metatarsal tubercle indistinct. Tibiotarsal articulation reached the snout when the hindlimb is kept parallel to the body. Skin smooth, upper parts green, with irregular distinct chocolate coloured patches with yellow dots at dorsal surface of body and limbs. Lower parts light and abdomen greenish.

Ecological notes: The species inhabits in the torrent habitat with high canopy cover. The vegetation is temperate evergreen mixed forest. Adults of *A. formosus* were collected from rock located at the bank of the fast-flowing stream with more than 90% of rock cover.

Distribution: This species is distributed in Assam and Sikkim, India and eastern Nepal (Günther 1875, Schleich and Kästle 2002, Dinesh *et al.*, 2009) (1200–2700 m).

Family: Rhacophoridae

Molecular analyses: The phylogenetic analysis based on 16S gene identified the six species of Rhacophoridae frogs: four species of the genus *Polypedates*, each from the genus *Raorchestes* and *Rhacophorus* respectively (Figure 37).

Polypedates samples from middle mountain (above 2000 m) of eastern Nepal (Maipokhari and Sulubung, Illam, Num and Seduwa, Sankhuwasava district) were genetically distinct than other species of the genus *Polypedates* and were identified as *Polypedates himalayanus* (see morphological description section for the identification). Specimens from Jhuwani, Chitwan, Nepal were identified as *Polypedates taeniatus* (see morphological description section for the identification). Based on the phylogenetic analysis, *P. himalayanus* was the sister taxon to *P. taeniatus* and the uncorrelated genetic divergence between them were 6.3%–5.9%.

Specimen collected from Belbari, Morang, Nepal (holotype locality of *Polypedates teraiensis*) and Baraha Kshetra, Sunsari, Nepal was assigned as *M. teraiensis* (for detail morphological characters: see the morphological description section for the details). *P. teraiensis* was the sister taxon to *P. leucomystax* (GQ204693), from Java, Indonesia and the uncorrelated genetic divergence between them were 3.1%–3.7%.

Polypedates samples from Chitrasari and Bharatpur, Chitwan district; Pokhara, Kaski district (central Nepal) and Mainapokhar, Bardiya district (western Nepal) were 94%–100% similar based on 16S data with the *P. maculatus* (GQ204694), from Sri Lanka (Meegaskumbura *et al.*, 2011). *P. maculatus* was the sister taxon to *P. pseudocruciger* (KU169984) from Kerala, India and the uncorrelated genetic divergence between them were 8.3%–10.8%.

A single specimen from Maipokhari, Illam, eastern Nepal was closely nested with the clade of genus *Raorchestes* group and was assigned as *Raorchestes annandalii*. *R. annandalii* was the sister taxon to *R. longchuanensis* (GQ285675), Yunnan, China and the uncorrelated genetic divergence between them were 5.0%.

A single specimen from Num, Sankhuwasava, eastern Nepal was closely nested with *Zhangixalus smaragdinus* (JX219411) from Motuo, Xizang, China (Li *et al.*, 2012). *Zhangixalus*

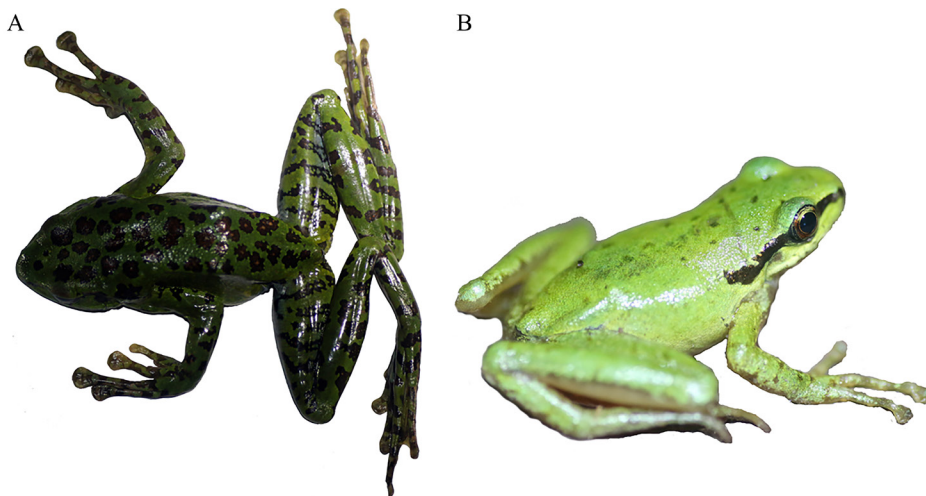


Figure 36 *Amolops formosus* in life. A) Dorsal view–adult male B) Dorsolateral view–juvenile. Photo by Janak Raj KHATTIWADA.

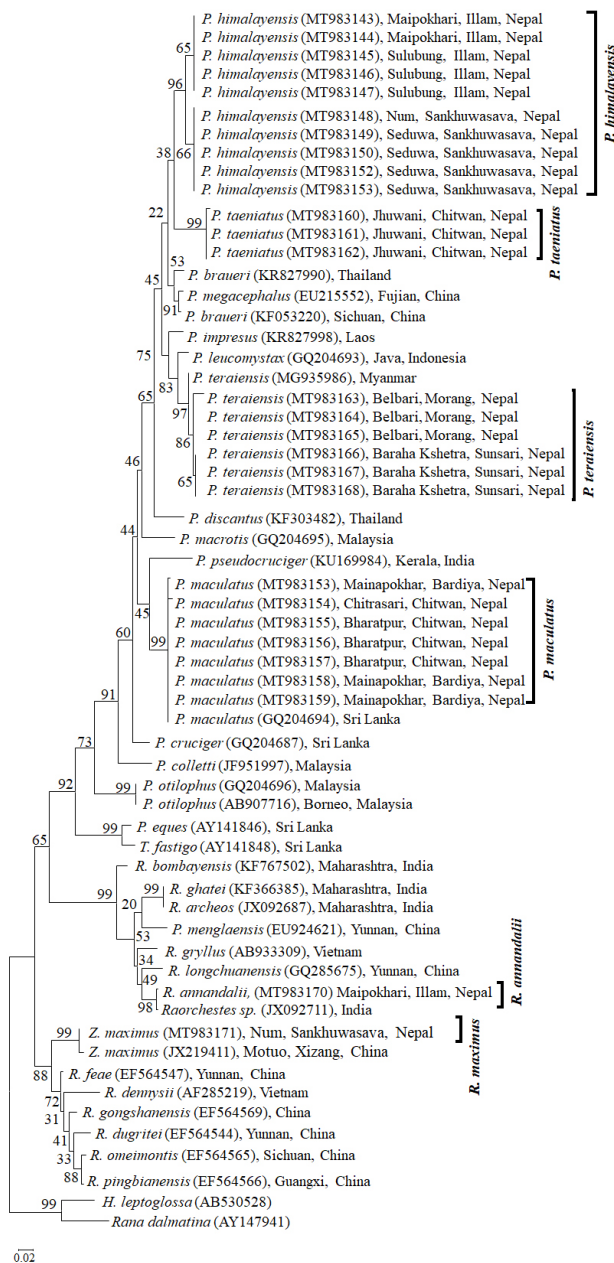


Figure 37 Maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the Rhacophoridae family. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

smaragdinus was the sister taxon to *Zhangixalus feae* (EF564547), Yunnan, China and the uncorrelated genetic divergence between them were 5.6%.

Genus: *Polypedates* (Tschudi, 1838)

Polypedates himalayensis (Annandale, 1912) (Figure 38)

Common name: Tree frog

Nepali name: Pahadi rukh bhyaguto

Description: Body size (SVL) female (60–63 mm) and male (49–55 mm).

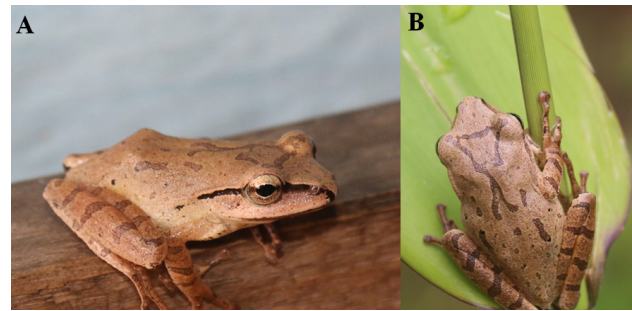


Figure 38 *Polypedates himalayensis* in life A: dorsolateral view; B: dorsal view. Photo by Janak Raj KHATIWADA.

Head wider than long, canthus rostralis slightly concave, nares closer to the tip of snout than eyes, internarial width shorter than interorbital width, interorbital width almost one and half times larger than width of upper eyelid, eyes are distinctly larger than diameter of tympanum, supratympanal fold distinct. Fingers long with lateral dermal fringe, relative finger length is $1 < 2 < 4 < 3$, fingertips with large adhesive pads with circum-marginal grooves. Subarticular tubercles round, formula 1, 1, 2, and 2. A large ovoid outer metacarpal tubercles present at the base of first finger and small round shaped outer metacarpal tubercle present at the base of the third finger. Toes with adhesive pads and webbed, relative toe length $1 < 2 < 3 < 5 < 4$. Subarticular tubercles round, formula 1, 1, 2, 3 and 2. Bean shaped inner metatarsal tubercle almost half size of the length of the first finger, small flat outer metatarsal tubercle. The tibio-tarsal articulation reaches to the tip of the snout. Dorsal and ventral skin smooth. Dorsal color variable. The dorsal mostly yellowish brown to dark brown. The interorbital and lower region of the head with triangular shaped brown marking. The dorsal body surface with irregular lines and dots. The loreal and temporal regions are yellowish brown. A thin black line passes dorsolaterally from the tip of the snout through supratympanic fold to the armpit, tympanum yellowish brown, dorsal surface of hand with 3 dark and two to 2–3 bands in fingers. The dorsal surface of thigh with 4 dark bands and trunk with 3 dark bands. Ventral surface is creamy white. Male with a pair of vocal sacs.

Natural history notes: Most of the species were observed in resting in the bushes just above the temporary or permanent pools and rice fields. This species is mostly nocturnal. Breeding is initiated from June to September. Females are much larger than males.

Distribution: Mostly distributed in eastern Nepal (1200–2400 m).

Polypedates taeniatus (Boulenger, 1906) (Figure 39)

Common name: Six-lined tree frog

Nepali name: Chhadharke rukh bhyaguto

Description: Body size (SVL) female (29–33 mm) and male (35–50 mm).



Figure 39 Dorsolateral view of *Polypedates taeniatus* in life. Photo by Janak Raj KHATTIWADA.

Head longer than wide, snout blunt and extended over the lower jaw, canthus rostralis somewhat concave. The interorbital distance larger than width of upper eyelid. Internarial and interorbital distance almost equal. The tympanum is distinct and about half size of eye diameter. Fingers free of webbing, relative finger length is $1 < 2 < 4 < 3$, fingertips with adhesive pads with circum-marginal grooves. Subarticular tubercles conical, formula 1, 1, 2, and 1. Toes webbed and toe tips with adhesive pads, relative toe length $1 < 2 < 3 < 5 < 4$. Subarticular tubercles conical, formula 1, 1, 2, 3 and 1. The inner metatarsal tubercle is oval and small lies at the base of first finger. The tibio-tarsal joint reaches the anterior corners of the eyes. The dorsal surface is smooth. The dorsal regions of the body and limbs are golden to reddish brown. There are six dark brown longitudinal stripes in the dorsal surface. A pair of dark brown stripe originated from posterior corner of eyes and extends to the groins at the dorsolateral sides. Then, a pair of broad and creamy white stripe originated from the nares over the tympanum to the

groin. Another pair of dark brown dorsal stripe originated from posterior corner of eyes lids and extends from lumber region to the groins. In the dorsal golden patch, there is a pair of light brown lines originated from tip of the snout extend over lumber region and fused at the cloacal region. The venter is a uniform creamish white. Male with subgular vocal sacs.

Natural history notes: Most of the species were observed in resting in the bushes just above the temporary or permanent pools and rice fields. This species is mostly nocturnal. Breeding is initiated from June to September. Females are much larger than males.

Distribution: Widely distributed in low land of Nepal (150–300 m).

***Polypedates teraiensis* (Dubois, 1987) (Figure 40)**

Common name: Common tree frog

Nepali name: Tarai rukh bhyaguto

Description: Body size (SVL) female 60–72 mm and male (49–55 mm).

Head wider than long, canthus rostralis concave, nares closer to the tip of snout than eyes, internarial width shorter than interorbital width, interorbital width almost two times larger than width of upper eyelid, eyes are distinctly larger than the diameter of tympanum, supratympanal fold distinct. Finger length is $1 < 2 < 4 < 3$, fingertips with large adhesive pads with circum-marginal grooves. Subarticular tubercles round, formula 1, 1, 2, and 2. Bean shaped outer metacarpal tubercles present at the base of the first finger and small round outer metacarpal tubercle present at the base of the third finger. Toes with adhesive pads and webbed, relative toe length $1 < 2 < 3 < 5 < 4$. Subarticular tubercles round, formula 1, 1, 2, 3 and 2. Oval shaped inner metatarsal tubercle larger than small rounded outer metatarsal tubercle. The tibio-tarsal articulation reaches to

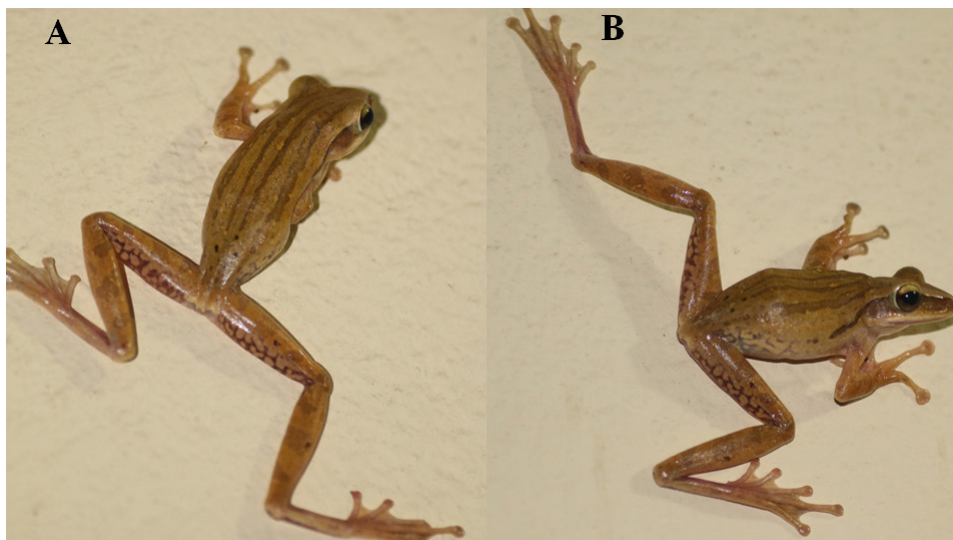


Figure 40 Dorsolateral view of *Polypedates teraiensis* in life. Photo by Janak Raj KHATTIWADA.

the tip of the snout. Dorsal and ventral skin smooth. The dorsal mostly yellowish brown. The loreal and temporal regions are yellowish brown. A thin black line passes dorsolaterally from the posterior corner of eye to the armpit, tympanum yellowish brown, dorsal surface of forelimbs with dark irregular bands, dorsal surface of thigh and trunk with 2 to 3 irregular dark bands, posterolateral side of the thighs with irregular brown spots, four thick dark line passes parallelly from the head to the groin region. The ventral surface is creamy white. Male with a pair of vocal sacs.

Natural history notes: Most of the species were observed in resting in the bushes just above the temporary or permanent pools and rice fields. This species is mostly nocturnal. Breeding is initiated from June to September. Females are much larger than males.

Distribution: Widely distributed in eastern Nepal (150–800 m).

***Polypedates maculatus* (Gray, 1834) (Figure 41)**

Common name: Chunam frog

Nepali name: Rukh bhyaguto

Description: Body size (SVL) female (48–52 mm) and male (37–47 mm)

Head length almost equal to width, canthus rostralis angular, nares closer to the snout than eyes, internarial width shorter than interorbital width, width of upper eyelid shorter than interorbital width, eyes are distinctly larger than diameter of tympanum, supratympanal fold distinct. Fingers long, relative finger length is $1 < 2 < 4 < 3$, fingertips with large adhesive pads with circum-marginal grooves. Subarticular tubercles



Figure 41 Dorsolateral view of *Polypedates maculatus* in life. Photo by Janak Raj KHATIWADA.

round, formula 1, 1, 2, and 2. A large ovoid metacarpal tubercle present at the base of first finger and flat outer metacarpal. Toes with adhesive pads and webbed, relative toe length $1 < 2 < 3 < 5 < 4$. Subarticular tubercles round, formula 1, 1, 2, 2 and 2. Single bean shaped inner metatarsal tubercle. The tibio-tarsal articulation reaches to the tip of the snout. Dorsal and ventral skin smooth. The dorsal colour variable from greyish or brownish or yellowish brown, W-shaped black band between the orbit, snout tip black. The loreal and temporal regions are dark brown to black. A thick black line passes dorsolaterally from the posterior corner of eye to the middle section of the belly, tympanum black, dorsal surface of forelimbs with dark irregular bands, dorsal surface of thigh and trunk with 2 to 3 irregular dark bands, posterolateral side of the thighs with irregular yellow spots bordered with brown, a thick dark bands present dorsolaterally on the each side of the trunk. Ventral surface creamy white. Male with a pair of vocal sacs.

Natural history notes: Most of the species were on observed in resting in the bushes just above the temporary or permanent pools and rice fields. This species is mostly nocturnal. In Nepal, the breeding is initiated from June to September. Sexual dimorphism, females are much larger than males.

General distribution: Widely distributed in central and western Nepal (150–800 m).

Genus: *Raorchestes*

***Raorchestes annandalii* (Boulenger, 1906)**

Common name: Himalayan bubble nest frog

Nepali name: Chhuschhuse bhyaguto

Description: Body size (SVL) between 15–19 mm (Schleich and Kästle, 2002)

Smallest frog of Nepal. The head length and width equal, snout pointed and slightly extended over the lower jaw, canthus rostralis distinct and round, loreal region slightly concave. The interorbital distance shorter than the width of upper eyelid, internarial distance almost equal to the interorbital distance, tympanum indistinct. Fingers free of webbing, finger with globular sucker pads and relative length $1 < 2 < 4 < 3$, subarticular tubercles flat, metacarpal tubercles absent. Toes with anteriorly truncated adhesive pads, webbing rudimentary, relative toe lengths of $1 < 2 < 3 < 5 < 4$, subarticular tubercles are flat, single inner metatarsal tubercle small and rounded. The tibio-tarsal articulation reaches the posterior corners of the eyes. Skin on dorsum and ventrum smooth, lateral sides the axilla, groin and ventral part of the femur granular. The dorsum is bronze greyish olive and ventrum is light bronze. Male with transparent vocal sac.

Ecological notes: This species is mostly found in the temperate forest. Species can be detected with short pitch whistle.

Distribution: This species is distributed in eastern Nepal and Darjeeling, India (900–2500 m).

***Zhangixalus smaragdinus* (Blyth, 1852)**

Common name: Giant treefrog

Nepali name: Kuthurka

Description: Refer to Blyth (1852) and Ohler and Deuti (2018) for the description.

Family: Salamandridae

Genus: *Tylototriton*

Molecular data. The phylogenetic analysis showed that *Tylototriton* samples from Nepal were 99–100% similar based on 16S data with the type series of *T. himalayanus* (KY800591), Maipokhari, Illam, Nepal (Figure 42) (Khatiwada *et al.*, 2015).

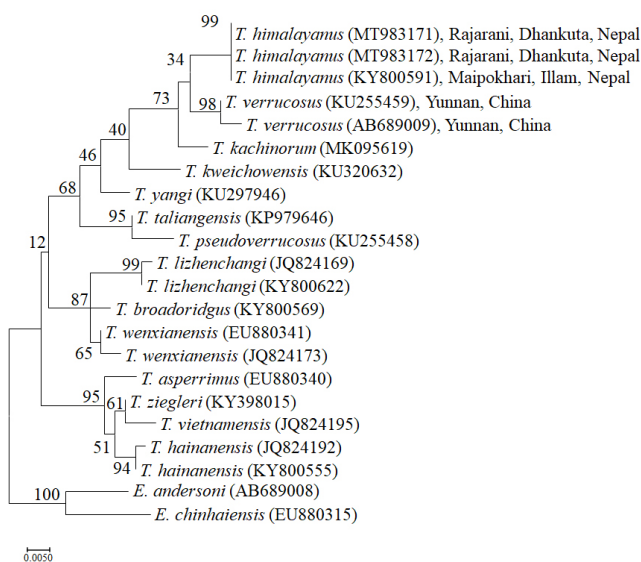


Figure 42 Maximum likelihood tree based on 16S gene sequence maximum likelihood tree derived from 16S rRNA sequences showing the phylogenetic relationships within the species of genus *Tylototriton*. Numbers present on branches are bootstrap support values for maximum likelihood. Genbank accession numbers are presented in parenthesis.

***Tylototriton himalayanus* (Khatiwada, Wang, Ghimire, Vasudevan, Paudel, and Jiang, 2015) (Figure 43)**

Common name: Himalayan salamander

Nepali name: Thakthake

Head was bluntly oval shaped; HL is slightly longer than HW. Snout is blunt, clearly extending beyond the lower lip. The bony ridges are present on either side of the head and are extended behind to eyes. Eye is moderate size with round pupils and black irises with large, granular upper eyelid. Skin of body is granular. Tail compressed laterally, with well-developed fin fold, a little shorter than snout–vent length. Uniform dark brown colouration in dorsal region but light brown colour in dorsolateral region, ventral surface has creamy colouration. Tail is comparatively lighter brown than body colour. The ventral



Figure 43 Dorsal view of *Tylototriton himalayanus* in life.

side of digits is rusty cream, but palms and soles are dull cream colour. The cloaca has a small longitudinal slit. Fingers and toes not webbed. Front and hindlegs muscular, with four fingers on the front legs and five toes on the hindlegs; relative lengths of fingers 3>2>4>1; relative lengths of toes 3>4>2>5>1.

Ecological notes: This species can be located nearby permanent and temporary ponds and agriculture land of temperate region.

Distribution: This species is distributed in eastern Nepal and North-eastern, India (900–2500 m).

Discussion

Studies on herpetological diversity of Nepal has begun since beginning of the 18th century, mostly during herpetofauna collection by European biologist (e.g., Brian Hodgson, Thomas Hardwicke, Hugh Falconer, Joseph Hooker H. W. Tilman, Oleg Polunin and Hermann Schlagintweit) (Günther 1858, 1860, 1861; Boulenger *et al.*, 1907; Leviton *et al.*, 1956; Swan and Leviton 1962). Later, Smith and Battersby (1953) examined the specimens collected from Langtang Mountain area in central Nepal and produced a brief checklist of amphibians and reptiles of Nepal. The first monograph of the herpetofauna of Nepal was published by Swan and Leviton (1962), which provided a basic overview of the taxonomy and zoogeography of Nepalese amphibians. In the late 1970s, the French herpetologist A. Dubois made a significant contribution to the biology of Nepalese amphibians. He and other collaborators collected more than 15,000 amphibian specimens from Nepal (Dubois, 1999) and described more than 10 new species (Dubois 1973, 1974b, 1974 1975b, 1977; Dubois and Matsui 1983; Dubois 1984, 1987). Later on, Nanhoe and Ouboter (1987) surveyed the amphibians and reptiles of Annapurna-Dhaulagiri region of central Nepal and compiled an updated checklist of herpetofauna based on both field observation and examination of museum specimens. They found 21 species of amphibians and 32 reptiles in the region. Further later, Zug and Mitchell (1995) and Mitchell

and Zug (1995) produced identification keys and a checklist of 11 amphibians and 42 reptiles of the Royal Chitwan National Park, Nepal, based on observation and examination of museum specimens. Similarly, based on three museum specimens, Das (1998) described a new species of *Hylarana chitwanensis* from the Chitwan district of Nepal. Schleich and Kastle (2002) published a guidebook of the herpetofauna of Nepal and shed light on the herpetology of Nepal. This book is still the best reference for the ecology and distribution of Nepalese amphibians and reptiles. Later, Shah and Tiwari (2004) also published a field guide for Nepal and updated information on the Nepalese herpetofauna. Although, there have been a large number of historical records and collections of specimens, however, there is still exists the uncertainty regarding the true amphibian biodiversity of Nepal. This study provided the current taxonomic status of Nepalese amphibians using both molecular and morphological approach. Based on our newly synthesized data (molecular and morphological), we have confirmed the occurrence of many new distributional records of species to Nepal. Our results detected 38 species of amphibians in the studied area, which covered more than 74% of the total amphibian species in Nepal. This study further highlights the occurrence of several unrecognized species and will increase this estimate of species diversity.

This study provides the new range extension of *Euphlyctis kalasgramensis* (Dicroglossidae), *Minervarya orissaensis* (Dicroglossidae) and *Hydrophylax leptoglossa* (Ranidae) to Nepal. Some of the species from genus *Amolops*, *Megophrys* and *Nanorana* remain unidentified due to paucity of specimens. Species from genus *Megophrys* are taxonomically complex and difficult to identify due to its small body size and cryptic nature. This the fact that the application of molecular technology in taxonomic studies during this study can reveal the existence of new species, identify the new range extension or presence of undescribed species in Nepal. Hofmann *et al.* (2017) also identified the highly divergent lineages of *Scutigera* frogs from Nepal Himalayas. Moreover, historical compilations of species by different researchers described above and recent field studies greatly improved the knowledge of Nepalese amphibians. Although species taxonomy using molecular methods in Nepal is increasing, future survey efforts targeting many inaccessible and poorly sampled areas would provide the accurate estimates of species richness of Nepal.

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